

STUDIES ON THE STRUCTURE AND FUNCTION OF
MECHANORECEPTORS IN THE STOMATOGASTRIC
NERVOUS SYSTEM OF SOME DECAPODA
CRUSTACEA

Malcolm Reginald Dando

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**Studies on the Structure and Function of Mechanoreceptors in
the Stomatogastric Nervous System of some Decapoda Crustacea.**

by

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Thesis submitted for the Degree of Doctor of Philosophy.

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DECLARATION

I hereby declare that the work recorded in this thesis has been carried out by myself, and that it is of my own composition. I further declare that it has not been submitted in any previous application for a higher degree.



I certify that Malcolm Dando has fulfilled the conditions laid down in the regulations for a degree of Doctor of Philosophy, under Ordinance No. 16 of the University Court of the University of St. Andrews and that he has accordingly qualified to submit this thesis for the Degree of Doctor of Philosophy.



Vitae

I was educated at Kingswood Grammar School, Bristol and attended university at St. Andrews where I graduated in Honours Zoology in 1965. The work described in this thesis was carried out between July, 1966 and March, 1969.

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Summary

- i. The stomatogastric nervous system of some of the decapod crustacea contains several small isolated ganglia. At least one of these ganglia is useful for the detailed investigation of the generation of a patterned central output controlling movement. A review of previous work shows that little is known of the anatomy and physiology of the sense organs innervating the foregut. This knowledge would be valuable for understanding the control mechanisms governing the foregut function because reflex mechanisms are probably important in this situation. Therefore the aim of the thesis was to investigate the mechanoreceptors innervating the foregut. Work was concentrated on these sense organs because they are simpler to record from than chemoreceptors and because they are probably involved directly in the control of movement.
- ii. The large amount of previous work on this type of sense organ in the higher decapods is summarised in an appendix. An attempt is also made to revise some of the terminology used in the study of these receptors, and recent developments are reviewed in some detail.
- iii. Two new major receptor systems innervating the foregut are described. The posterior stomach nerve (p.s.n.) contains a group of about 80 neurones which innervate the posterior of the gastric

mill. These neurones respond to normal movements of the mill. Changes in the input from the receptors affect the output from the stomatogastric ganglion. The other system (MPRs) consists of three distinct organs totalling about 40 neurones which monitor normal movements of the structures around the mouth.

iv. Suggestions are made for further specific work on these two receptor systems. What is now known of the sensory innervation of the foregut is summarised and suggestions are made for completing this knowledge.

v. Further possible work on the ganglia and the role of the associated mechanoreceptor organs is reviewed. Finally work on other problems related to the studies reported here is discussed.

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Part I. Introduction

Section 1.1. Object of Study

A central question in the study of nervous systems during this century has been the relative importance of centrally generated and peripherally induced mechanisms in the regulation of behaviour (see, for an extended discussion, *Mechanisms of Animal Behaviour*, Marler and Hamilton, 1967). The control of animal movement has been a major aspect of the general problem because activities like locomotion are readily observable and can withstand much experimental manipulation.

Over the last decade a small number of arthropod preparations have been analysed in sufficient detail for the neuronal mechanisms to be reasonably clear. Examples of these analyses are locust flight (Wilson, 1968) and crayfish abdomen position (Kennedy et al., 1967). Arguing mainly from this evidence many workers now think that the predominant feature of the control of movements in the invertebrates is centrally determined sequences of motor impulses, and that the control reflected from the periphery is limited (e.g. Horridge, 1968). Nevertheless mechanisms directly involving sense organs may sometimes be important. For example, Usherwood, Runion and Campbell (1968) after a careful study on locusts conclude that leg

reflexes involving the femoral chordotonal organ and femoral muscles appear to play a very significant role during postural and walking activity. Unfortunately all of these studies have suffered to some extent because the central component lies within the central ganglia and it is therefore difficult to investigate the basic problem, the inter and intra-cellular mechanisms which generate the central output (Wilson, 1966).

The work of Maynard (1966, 1967) is therefore important. He has reported studies on a preparation which is of great value for detailed analysis of the central generation mechanisms. This preparation is the stomatogastric ganglion of the (reptantian) decapod crustacea. The small isolated ganglion of 30-35 neurones is concerned, together with the slightly smaller oesophageal and somewhat larger commissural ganglia, in the control of foregut movements. It can produce a complex, patterned output in response to an unpatterned input and it is possible to study the activities of the cells in great detail. The other two ganglia are also accessible for investigation.

In this situation reflex control may also be important, but the sense organs of the foregut of the decapods are not well known. Therefore the object of this work was to describe anatomically and physiologically the major internal mechanoreceptors in the (reptantian) decapod foregut with a view to using this information subsequently

in the study of the ganglia and the mechanisms of control of the gut. The work was concentrated on internal mechanoreceptors because of the ease of recording from these receptors and their probable direct involvement in posture and movement of the foregut (i.e. proprioception). Additionally this was of interest because the study of these sense organs in the decapods has to a large extent been confined previously to the abdominal MRO and the walking leg receptors.

Because of the large amount of recent work on proprioception in the decapod crustacea an appendix (p. 88) is devoted to a review of this subject. Section 1.2 of this introduction completes the background information with a review of previous relevant work on the stomatogastric nervous system in this group. Part 2 of the thesis is concerned with the experimental work and part 3 with a discussion of the work in relation to previous information and the specific problem of the control of the foregut.

Section 1.2. Previous work on the Nervous System Innervating
the Foregut of the Decapoda Crustacea (Reptantia).

1.2.1. Anatomy

i. Gut Anatomy

The anatomy of the foregut of a typical reptantian decapod is

described in most textbooks of invertebrate zoology. There is not a great deal of variation in the general plan through the sub-class. The foregut of Nephrops, for example, was described in detail by Yonge (1924) in the first volume of the Journal of Experimental Biology. A short vertical oesophagus leads to a spacious cardiac stomach. In the dorsal posterior region of the cardiac stomach is a gastric mill equipped with two lateral and a medial tooth for breaking up food. Behind the gastric mill lies a filtering apparatus called the pyloric stomach and this leads to the mid-gut.

A complex system of mainly bilaterally arranged muscles moves the gut to effect ingestion, trituration and food passage. There is some variation in the muscle systems of the three groups, the Macrura in general having the least developed foreguts. The same muscle plan, however, is recognisable throughout the sub-class. For example Pearson (1908) gave a complete description of the muscles in Cancer pagurus. These muscles must contract in well defined temporal sequences in order to effect the functions of the foregut.

ii. General plan of nerves

The early work started in the last century by Audouin and Milne Edwards is summarised by Keim (1915) and Blass (1941). Two later investigations form the basis of our present more detailed knowledge.

These are the methylene blue studies of Allen (1894) on Homarus vulgaris and Orlov (1926-30) on species of Astacus. The work of Heath (1941) on the brachyuran Pugettia producta also provides some valuable details.

Whilst the endings of the nerve fibres are highly variable, the course of the major nerves is constant. The findings of Orlov are summarised in figures 1.2.1a. and 1.2.1b. Two bilaterally arranged commissural ganglia (cg) lie on the oesophageal connectives in front of the sub-oesophageal ganglion (sb.g). Running forward from each commissural ganglion are two nerves, the inferior and superior oesophageal nerves (io, so). These two paired nerves unite on the front of the oesophagus (o) to form the curiously elongate medial oesophageal ganglion (og). A small ventral medial nerve (vm) connects this ganglion to the supra-oesophageal ganglion (sp.g). From the oesophageal ganglion a medial stomatogastric nerve (st) runs up over the foregut to the stomatogastric ganglion (sg) which lies between the anterior gastric muscles (agm). A small medial dorsal nerve (dm) connects the stomatogastric nerve and the supra-oesophageal ganglion. From the stomatogastric ganglion a medial dorsal ventricular nerve (dv) runs posteriorly and divides into the paired lateral ventricular nerves (lv). These two nerves diverge to pass around the

FIGURE 1.2.1a.

Diagrammatic side view of the anterior cephalothorax of Astacus to show the stomatogastric nervous system. Redrawn from Orlov (1926a). Lettering is explained in the text. A pyloric sensory cell is shown on the posterior of the foregut.

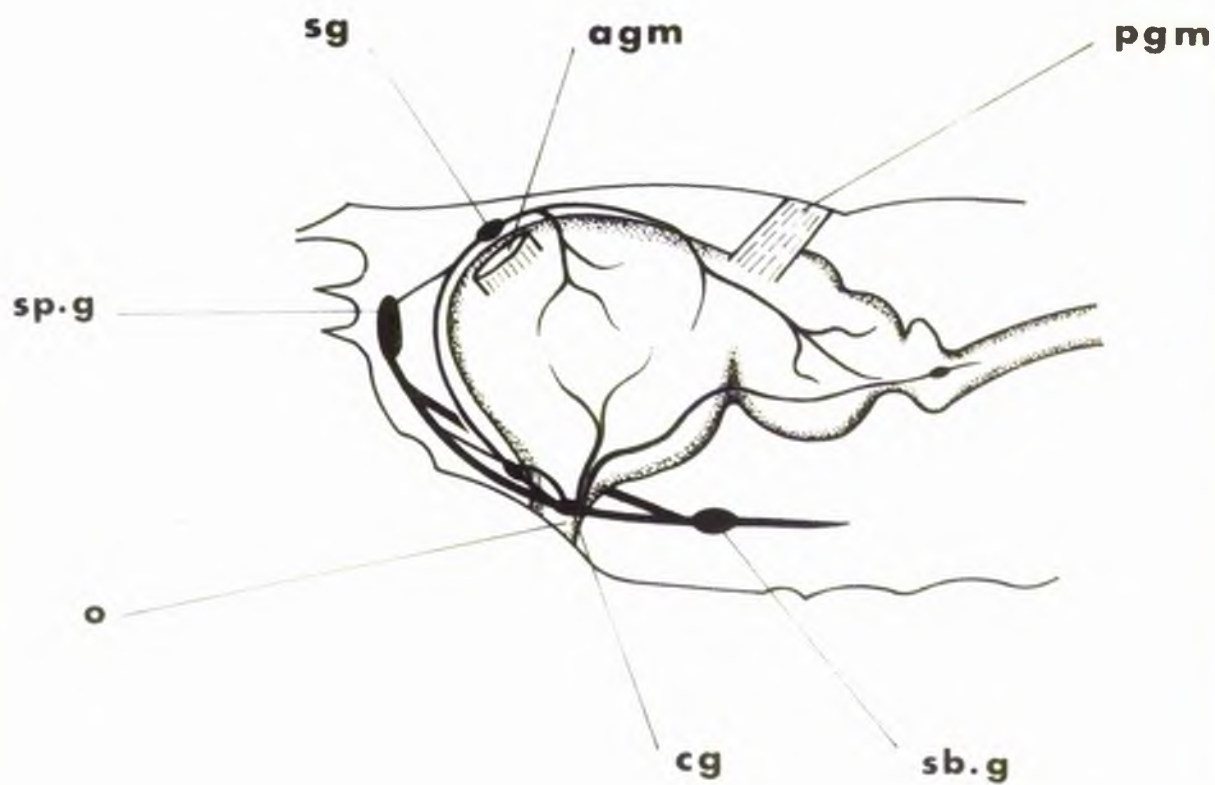
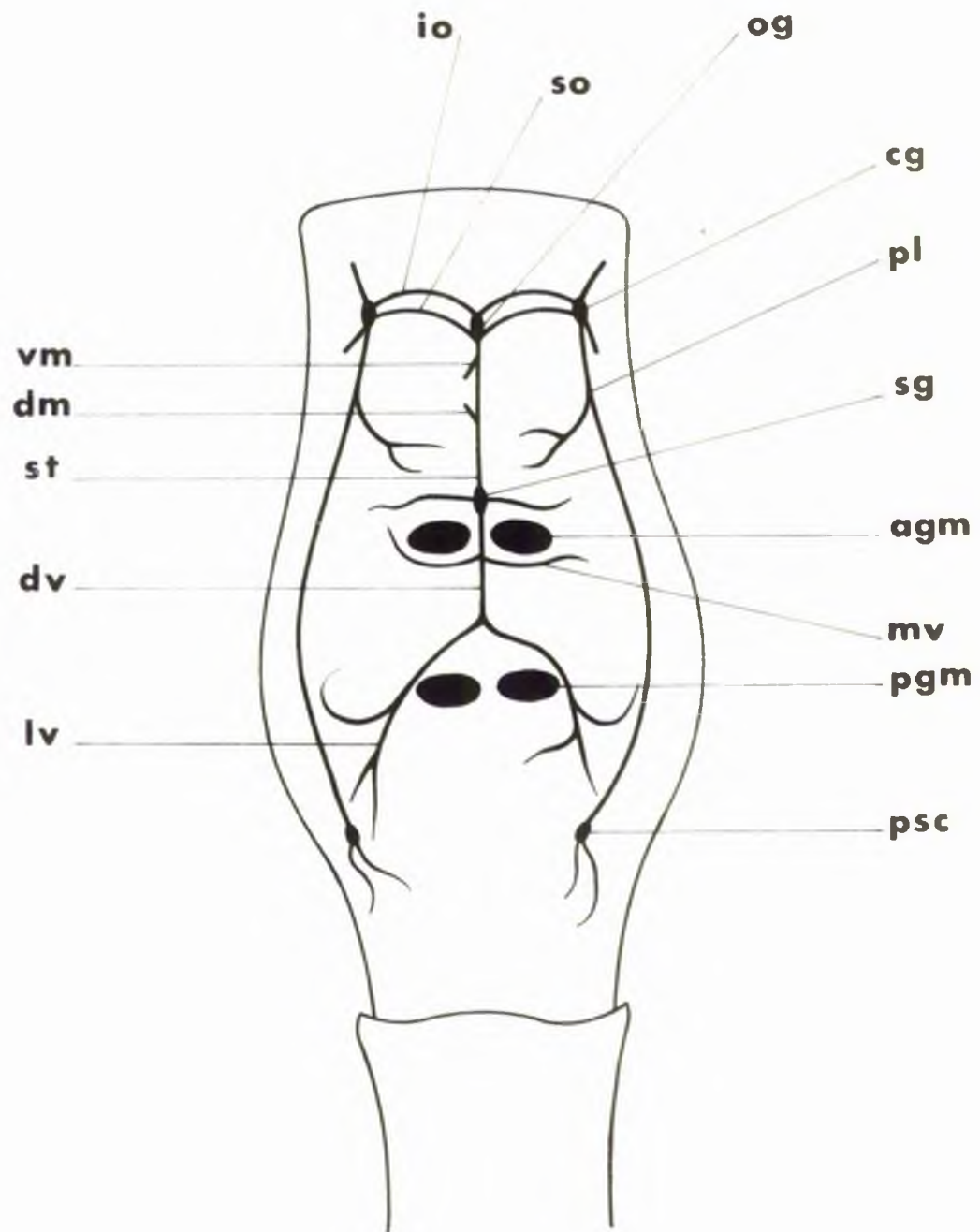


FIGURE 1.2.1b.

Dorsal view of the flattened stomatogastric nervous system of Astacus. Redrawn from Orlov (1926a). Lettering is explained in the text.



posterior gastric muscles (pgm) and on to the sides of the foregut. Several other bilaterally arranged nerves (for example the medial ventricular nerve, mv) run from the ganglion to the muscles of the gut.

Another large paired nerve, the postero-lateral nerve (pl), runs from the commissural ganglion along the underside of the foregut and then on to the mid-gut. (I have found that in Homarus this nerve sometimes may not run directly from the commissural ganglion but from the superior oesophageal nerve). A number of other nerves run from the commissural ganglion to innervate the oesophagus and lower cardiac stomach. Additionally Heath described the full course of another large paired nerve in the crab Pugettia producta. These nerves ('s' in his terminology) are called the posterior stomach nerves (p.s.ns.) here. The nerves terminate peripherally around the insertion of the posterior gastric muscles on each side of the gastric mill. From this point each nerve runs in an antero-lateral direction to the region above the mandible. It then descends ventrally to run back, usually fused with the mandibular nerve, into the thoracic ganglion. This nerve is probably the hepatic or terminal nerve of many previous authors.

iii. Ganglia

The neuronal arrangement of the ganglia of the foregut has been

beautifully described by Orlov (1927, 1929). As an aid to further work Bullock and Horridge (1965) presented a detailed review of Orlov's findings. The commissural ganglion, although it is smaller, has the normal structure of a central ganglion with a neuropile surrounded by a cortex of cell bodies. The oesophageal ganglion is a diffuse structure, with cell bodies situated mainly at the junction of the two inferior oesophageal nerves and the ventral median nerve. The neuropile is spread along from this junction to the junction of the two superior oesophageal nerves, but it is mainly concentrated at the latter more dorsal position.

Two major points from Orlov's work need to be noted here. He concludes that the sensory pathways of the foregut are directed into the commissural ganglion, and that the stomatogastric ganglion does not possess the anatomical structure which permits independent reflexes. Orlov states that the stomatogastric ganglion contains the following three elements:

- (a) Efferent fibres of central origin which give off collaterals in the ganglion and then pass on to the gut muscles.
- (b) Axons of the two cardiac sensory cells (see iv.B.) which do not synapse in the ganglion.
- (c) The 30-35 motor neurones which have branches in the neuro-

pile and send axons to the gut muscles.

It is usual for the bilaterally symmetrical gut muscles to be innervated from the two branches of a motor neurone which has divided near the stomatogastric ganglion.

It should be noted in passing that monoamines occur in the stomatogastric ganglion and that these substances may act as transmitters between the ganglion cells or between the motor neurones and the muscles of the gut (Osborne and Dando, 1969). Studies of the fine structure of the ganglion give results which are in agreement with the occurrence of monoamines in the ganglion, and also show that the stomatogastric nerve contains a wide range of fibre sizes with groups of numerous small fibres. (J. Dando, personal communication).

iv. Sensory systems

Orlov (1926a, b) gave a detailed account of the peripheral sensory cells on the oesophagus, stomach and midgut of Astacus. There are some points of difference from the earlier account of Allen (1894) for Homarus.

A. Oesophagus

a. Uniterminal nerve cells

These cells are distributed under the epithelium and their peripheral processes penetrate through this tissue. The axons from

the cells run into the commissural ganglia. Orlov distinguishes: Groups of 3-5 cells scattered on the oesophagus, and two groups of at least 100 cells situated to the right and left of the stomatogastric nerve.

Allen deals only with the latter groups of cells. He states that the peripheral processes of these cells penetrate into the lumen of the gut and that their axons pass into the superior oesophageal nerve and thus to the commissural ganglion. Allen proposed that these cells were organs of taste, a suggestion supported by Orlov.

Moulins and Dando (unpublished) have confirmed that the large cell groups in Homarus and Astacus do have peripheral processes which penetrate the cuticle. The groups occur on each side of the anterior medial lobe of the oesophagus (see Yonge, 1924) at the junction with the cardiac stomach. The fine structure of the dendrites of the neurones is strikingly similar to that of insect hypopharyngeal chemoreceptors (Moulins, 1968). Unfortunately, it has not yet been possible to record satisfactorily from these neurones in order to confirm that they are chemoreceptors.

b. Multiterminal nerve cells

According to Orlov multiterminal neurones are distributed widely over the free surface of muscles and the dendrites ramify diffusely in the subepithelial connective tissue. Once again the axons of these cells

run into the commissural ganglia. Allen also described cells, somewhat larger than those in the presumed chemoreceptor organs. These were bipolars with long dendrites. Orlov states that this was a mistake and that the long dendrite breaks up and does not end in one terminal. I have not found many of these larger cells on the oesophagus but my observations support Orlov's view.

B. Stomach

a. Cardiac 'k' cells

In Astacus two curious cells are situated near or in the stomatogastric ganglion. Each cell has two peripheral processes which run one on each side of the gut. The central process does not join the neuropile of the ganglion but passes undivided to the oesophageal ganglion. Here it divides into two processes which pass to the commissural ganglia. The form of these cells resembles the multiterminal cells scattered on the gut musculature. Allen also described similar cells in Homerus.

b. Pyloric cells

Three cells situated on each side of the gut in the region of the pyloric stomach send peripheral processes to the mid and hind gut. These cells have the same general form as the multiterminal neurones. The central processes run to the commissural ganglia in the postero-

lateral nerve. In both Nephrops and Homarus I have seen small numbers of bipolar (multiterminal) nerve cells on the pyloric region of the gut. Because of the numerous anastomoses between the nerves in this region it is not clear whether the axons from these cells run back into the commissural ganglion or into the stomatogastric ganglion via the lateral ventricular nerves.

c. Subepithelial plexus

The connective tissue surrounding the ossicles of the masticatory apparatus is rich in diffuse dendritic terminations. Orlov speculates that these dendrites come from the cardiac cells and from the cells of the oesophagus. Allen states that a small number of his larger bipolar cells were scattered over the whole foregut and not confined to the oesophagus. It should also be noted that Heath's nerve 's' runs into this area and could be contributing to this plexus if it contains sensory cells. Heath does not describe sensory cells in the nerve or on the gut. His work is more concerned with the general layout of the nerves.

d. Innervated hairs inside the stomach

Ringel (1924) described some of the hairs inside the stomach as innervated sensory organs. Yonge (1932) states that the hairs inside the stomach are definitely not innervated. This subject has not been

investigated with modern techniques.

1.2.2. Physiology

Very little information is available on the physiology of this part of the crustacean nervous system. The mode of action of the mandibles, labrum, oesophagus, stomach and gastric mill is virtually unknown. Old reports are discussed in section 3.1.1. Recently in addition to the work of Maynard on the stomatogastric ganglion and the work on sensory systems dealt with in this thesis there has been only one other investigation by Larimer and Kennedy (1966) on the 'k' (cardiac) sensory cells described by Orlov.

1. Stomatogastric Ganglion

Maynard has reported on two aspects of the generation of the patterned output by the stomatogastric ganglion in response to stimulation of stomatogastric nerve input to the ganglion.

A. Homarus stomatogastric ganglion; relationships between units in the lateral, medial and anterior ventricular nerves,

Maynard (1966) reported a study in which he used extracellular wire electrodes to monitor the effects on four units of preganglionic stimulation. Figure 1.2.2. shows the experimental arrangement and the form of the cyclic response. Table 1.2.1. shows the units, nerves and their destinations. After confirming that the activity he was

FIGURE 1.2.2.

Diagram of isolated stomatogastric preparation arranged for recording. Recording electrodes were placed on (a) anterior ventricular nerve, (l) lateral ventricular nerve, and (m) median ventricular nerve. Recording (sg) or recording and stimulating electrodes (S) were placed on stomatogastric nerve. Traces above preparation diagram the form of activity recorded; a single unit from a and m, two units from l.

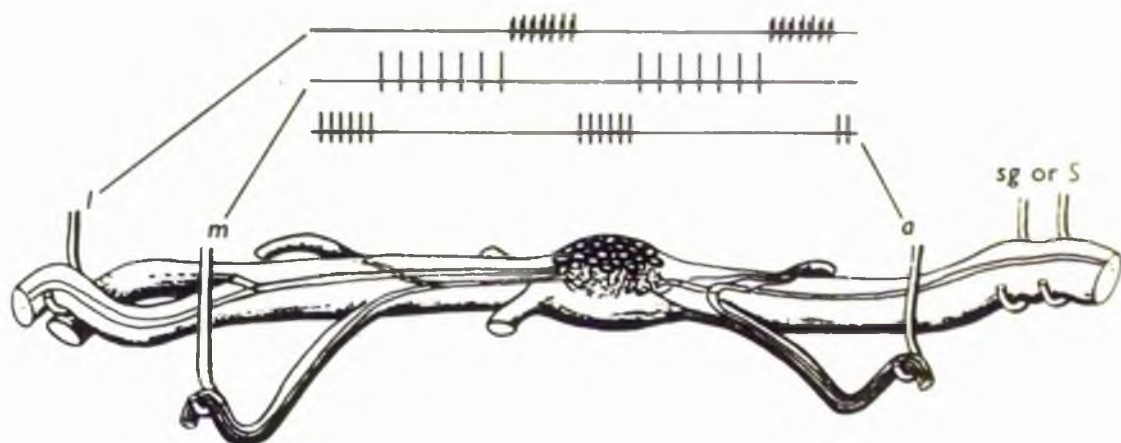


Table 1.2.1.

Homarus stomatogastric ganglion: relationships of units.

nerves and muscles

<u>Unit</u>	<u>Nerve</u>	<u>Destination</u>
a	anterior ventricular	ventro-lateral cardiac muscle
m	median ventricular	lateral gastric dilators
l (two neurones)	dorsal and lateral ventricular nerves	unknown

recording occurred in minimally dissected animals he asked one question:

'What kinds of interaction within the stomatogastric ganglion produce this cyclic patterned output?'

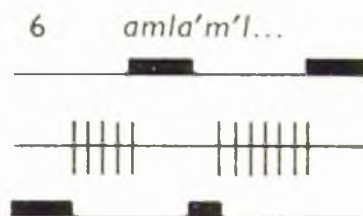
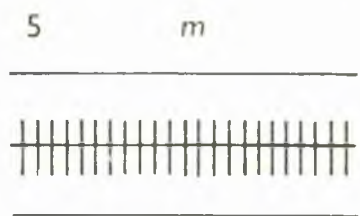
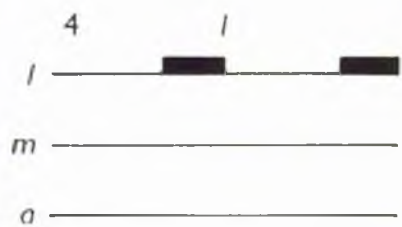
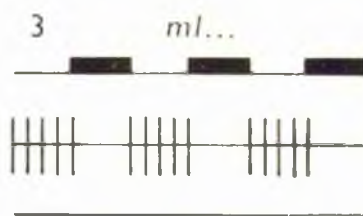
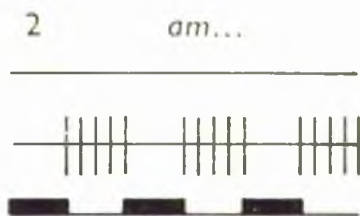
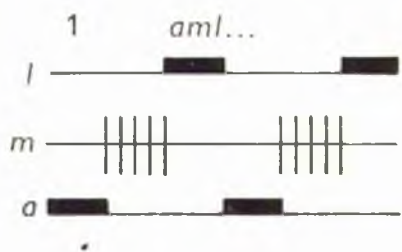
Figure 1.2.3. shows the range of variations in the output which he found. A-m and m-l cycles occur without a gap left by the missing element. Discharge of the l unit in bursts in the absence of other activity in a or m indicates that the burst formation is not dependent on the interactions considered here (see B). In contrast bursts or interrupted discharges in m do seem to require activity in either a or l. In the 6th variation short bursts of a alternate with long bursts in the next cycle, the phase relations between a and l and the cycle frequency are constant but the time gap between a and l is filled with m activity. Such variations indicate that the precise phasing and sequence of the cycle, with little or no overlap, depends upon interactions between the units not upon an external pattern generator.

Maynard then went on to show the effects upon the pattern activity of variations in stimulus strength and frequency of the presynaptic priming. Increasing stimulus frequency increased cycle frequency and both instantaneous and average frequencies of elements a and m (whilst l burst remained relatively constant). It seems that the

FIGURE 1.2.3.



Diagram of variations of cyclic activity recorded from anterior (a), median (m) and lateral (l) ventricular nerves. Horizontal thickenings represent bursts of repetitive spikes in a or l as indicated; time runs from left to right. Three possible combinations, a alone, al ..., and alm ... have not been observed as yet.



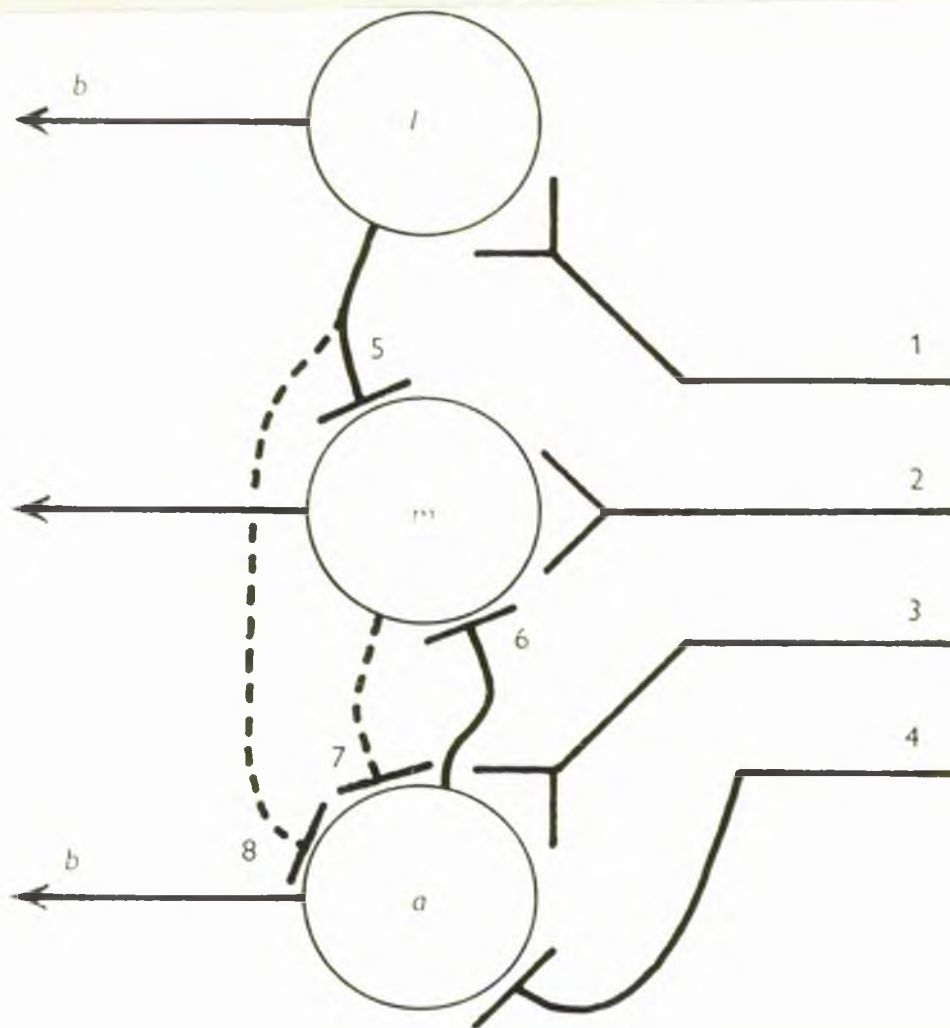
excitability of all the elements increased but overlap did not occur and increases in cycle frequency and duration of a burst activity occurred at the expense of the duration of the m burst. Increasing stimulus strength (e.g. no. of afferents) at a constant frequency produced different results. With low level priming only l units discharge, firing in bursts. Then the frequency of l bursts increases and the m begins to fire between the bursts. With stronger priming the frequencies of both l burst and m unit activity increase and a unit bursts appear. Again both the increases in cycle frequency and a bursts occur at the expense of m burst duration. Maynard states that in all probability a and l unit activity is associated with inhibition of the m unit.

Figure 1.2.4. summarises the network of interconnections suggested by the experiments. Inputs 1-3 are thought to be independent because of the different thresholds shown to stimulus strength, however they probably represent more than one channel. The inhibitory priming channel to a is demonstrated by the fact that a discharges only at the end, and never during, strong high-frequency priming.

Given active elements l, m and a the critical interconnections for the production of cyclic activity are 5-8. All these are inhibitory.

FIGURE 1.2.4.

Stomatogastric ganglion, suggested interconnexions necessary for cyclic activity. a, l, and m represent neurone or neurones sending axons in the anterior, lateral, and median ventricular nerves respectively. The small 'b' above the output arrows of units a and l indicates that these discharge in bursts that are not determined by the network diagrammed here. Connexions 1-4 represent afferents from the stomatogastric nerve which synapse with stomatogastric neurones. 1-3 are excitatory, and each connexion, e.g. 1, or 2 or 3, probably contains several fibres. Connexion 4 is inhibitory. Connexions 5-8 presumably occur within the ganglion neuropile between the units shown, and all are inhibitory. The evidence for connexions 5 and 6 is relatively direct. Connexions 7 and 8 are inferred from certain aspects of the cyclic pattern (see text) but have not been demonstrated directly. The connexions shown are the simplest possible to account for the observations, but should not be taken to imply the absence of additional complexities. For example, the possibility of inhibitor interneurones between l and m, or a and m in place of the direct connexions shown cannot be excluded with present evidence.



Connections 5 and 6 from l and a to m are well demonstrated by the inhibitions of the m unit activity. Connection 7 is suggested by the a m l a' m' l cycle and 8 seems necessary to account for the failure of the discharges of units l and a to overlap. Maynard concludes that: 'The integrative capacity of the stomatogastric ganglion appears to be on order of magnitude beyond that of the cardiac ganglion. In addition to synchronous bursts, the system is capable of sequential or ordered discharges reminiscent of respiratory or locomotory cycles found in more complex ganglia. Although analysis has only begun, the production of these patterns does not seem to involve a single, monolithic mechanism controlling all aspects of the discharge. Rather, to some extent it seems to take already complex activity, such as the burst of the l elements, and weld it together with other systems and units to produce the final pattern. Even in such a stereotyped and simplified system as this therefore, the principle of a hierarchy of organisation, or integration by levels, seems basic.'

B. Scylla stomatogastric ganglion: relationships between units in the lateral ventricular nerves

Again in this investigation Maynard and Burke (1966) and Maynard (1967) were interested in the mechanisms which generated patterned activity within the ganglion when the stomatogastric afferents were stimulated.

However here they used intracellular techniques to study the activity of units also recorded in one lateral ventricular nerve. Unfortunately no figures are available as only an abstract of a talk and a short note are available. Figure 2.1.15. shows the activity found often in the nerves from the stomatogastric ganglion of Cancer pagurus. It appears possible to equate this recording to some extent with Maynard's description. He states that group A fires first. It contains 4 neurones, two with large potentials and two with small potentials. Following the A burst there is a short silent period before the single B neurone discharges. As the B-neurone stops, small s-potentials from at least 4 neurones begin to appear. These increase to a steady level and then taper off abruptly as the A burst begins again. The sequence A, B, s, is stable and often continues for long periods with little change. In other preparations, the B-element may be irregular or absent without markedly changing the time course of the A-s alternation. The top trace of line B in figure 2.1.15. (p. 50) shows activity in the dorsal ventricular nerve. The trace starts with a group of small units, then a large unit fires. After this burst there is a distinct silent period followed by one larger unit bursting. Then the small group fires again. I think that the largest unit is probably B, the next largest part of the A group, and the small group the s units.

Examination of line C (lateral ventricular nerves) shows that the medium unit is in fact double (two units firing almost synchronously) and that another two smaller synchronous units sometimes fire at the same time. This tends to confirm these units as group A of 4 neurones. Figure 1.2.2. shows Maynard's units in the Homarus stomatogastric ganglion. It will be seen that the l unit is in fact two synchronous units, suggesting that they are equivalent to the larger units of the A burst in Scylla. This view is supported by the timing of the large unit in the lower trace in line B of figure 2.1.15. This trace shows the recording from the outer lateral nerve (median ventricular nerve). The large unit is probably equivalent to Maynard's m unit as it is the only one of the two which sometimes fires regularly without bursting (compare figures 1.2.3. and 2.1.16). This unit in line B of figure 2.1.16. always fires just before the A burst of the dorsal ventricular nerve. This is the correct m l timing to fit figure 1.2.2. It appears therefore that the A unit discussed here is equivalent to the l unit discussed in subsection A.

It is difficult to discuss the experimental work without more information but the conclusions are clear enough in themselves.

'Of the nine cells involved the four A-neurones coupled electrotonically provide the timing and organizing signal for the pattern.

Whether the A-burst involves an active slow membrane response or not

is unclear, but it does appear to require the observed reciprocal coupling. The single B-neuron is under inhibitory bombardment from two sources, directly from the g-neurons and indirectly from the A-neurons. It is released from inhibition only following the A-burst and at that time may reach sufficient depolarization to spike. The B-spikes evoke IPSP in some of the A-neurons so that reciprocal inhibition exists between A and B cells. This insures that either A or B elements, but not both, are active, but does not play a significant role in determining the A, B, g sequence. The 4+ g-neurons inhibit the B-element. They serve two functions, (1) they keep the B-cell inactive so that it does not interfere with the start of the A-burst, and (2) because of inhibition during and after the A-burst, they allow post-inhibitory excitation in the B cell to develop. We do not know whether the g-neurons react more directly with the A-cells, but at this time we suppose that they do not'.

They add,

'that all possible formal patterns of interconnection between two elements exist in the ganglion.

1. Reciprocally excitatory, A-A cells, leading to positive feedback and overload.
2. Reciprocally inhibitory, A-B cells, leading to a bistable system in

which either one or the other may be active at a given time, but not both.

3. Negative feedback, leading from the A elements, through inhibition of the inhibitor, to effective excitation of the inhibitory B-cell. According to parameters of the elements, this may lead to a stable or an oscillatory response.'

In addition to further work on the ganglion itself, the questions now to be asked are what is the nature of the presynaptic input to the stomatogastric ganglion and what are the effects of the output on the muscle system.

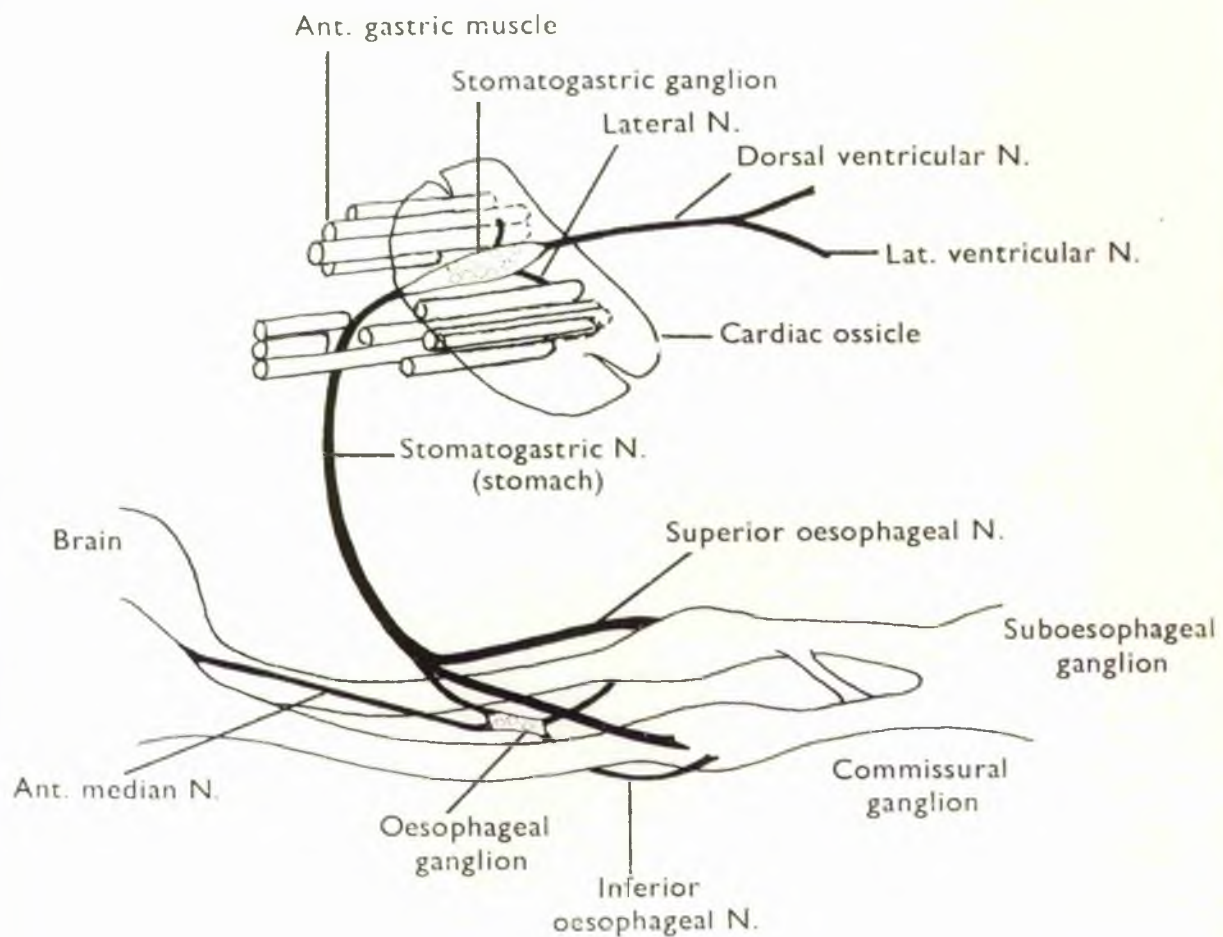
ii. Cardiac Sensory Cells

Larimer and Kennedy (1966) studied the stomatogastric nervous system of Procambarus clarkii. This system is shown in figure 1.2.5. They began by recording from the cut end of one superior oesophageal nerve. Normally there was a complex but stereotyped sequence of discharges in about three axons. This pattern was repeated at a frequency identical to that of normal stomach contractions. These afferent signals are therefore a centrally directed report of the complex movements of the anterior digestive tract.

The most prominent element of the discharge was a fibre which fired rhythmically at low frequencies in preparations in which only

FIGURE 1.2.5.

Diagram of the stomatogastric system of Procambarus clarkii.



the peripheral connections to the stomatogastric ganglion were left intact. They worked on this element. The regular discharge of the unit changed to give a phaso-tonic response to imposed ramp stimuli. Natural stimulation often broke the discharge up into discrete bursts. Identical recordings of this unit could be obtained from the stomatogastric nerve or from either superior oesophageal nerve. Clearly the cell has a branch down each superior oesophageal nerve. Cutting the dorsal ventricular nerve had no effect on the cell or its response to mechanical stimulation. Cutting one of the lateral nerves (probably equivalent to NGA of Maynard, 1966) caused transient increases in frequency. Cutting both lateral nerves produced variable results such as prolonged regular discharges which suggest that the cell was capable of maintaining autogenic activity with the ganglion totally isolated. It was, however, clear that the lateral nerves contained the elements responsible for the mechanical responses of the cell, since cutting either one eliminated the discharge that could be evoked by manipulation of the ossicle on that side. In subsequent experiments recordings were made from both lateral and both superior oesophageal nerves in various combinations. These recordings confirmed that the discharges were synchronous and that they could be reset by stimulation of these nerves. Stimulation of the oesophageal connectives failed

to reset the discharge rate and this indicates that the neurone has terminal synapses in the commissural ganglia.

Larimer and Kennedy state that the unit under analysis was a four-branched cell with its soma in the stomatogastric ganglion and processes in each lateral and each superior oesophageal nerve, that it was capable of autogenic discharge, and that it responded to mechanical stimulation of the anterior stomach. This unit fits very well with the sensory cells described by Orlov in the stomatogastric ganglion (k' cells) and Larimer and Kennedy regard these elements as identical.

Their subsequent work showed that the side of the cardiac stomach undergoing the most distortion causes its dendrite of the cell to fire faster than the other dendrite and that this frequency invades all the other branches. (The dendrites carry action potentials, see appendix). Functionally therefore the unit serves to produce a balanced output from an unbalanced input. The output frequency to each commissural ganglion is identical, and equal to the most active receptor terminal. They did not investigate the reflex effects of the input but suggest that it influences the motor output to the anterior gastric muscles and that its motor influence is bilaterally distributed. Therefore the entire reflex would act as a mixing circuit that could balance accidental

asymmetries in the action of the relevant muscles. They suggest that given this function the cell represents the most economical way of doing the job. It should be noted that they do not rule out the possibility that the cell has connections in the stomatogastric ganglion.

1.2.3. Summary of Previous Work

- a) The general plan of most of the major nerves in the anterior stomatogastric system is known, but individual variations and frequent anastomoses prevent effective anatomical tracing of the peripheral endings of many nerves.
- b) The neuronal arrangement of the ganglia in Astacus is well described. The commissural ganglia appear to be the only place where sensory information is directed. It is known that this ganglion produces a complex temporally patterned output but no detailed investigations have been made. The function of the oesophageal ganglion is unknown. The stomatogastric ganglion has been the subject of detailed investigation. It seems to control the activities of much of the stomach but its own activities are dependent on presynaptic information from the CNS. The nature of this input is not known.
- c) Several sensory systems are described anatomically and one interesting system has been analysed physiologically. There are,

however, numerous gaps in our knowledge.

d) The layout of the major muscles is known but no physiological information is available.

e) The mode of action of the foregut is not well described.



Edena Grove

Bone

TIN SEED - AIR DRIED

2

Part 2. Experimental

Section 2.1. The Posterior Stomach Nerve

2.1.1. Introduction

Heath (1941), who worked on the kelp crab Pugettia producta, is the only author to have fully described the course of one of the larger paired nerves ('s' in his terminology) in the stomatogastric nervous system. These nerves terminate peripherally around the insertion of the posterior gastric muscles on each side of the gestic mill. From this point each nerve runs in an antero-lateral direction to the region above the mandible. It then descends ventrally to run back, usually fused with the mandibular nerve, into the thoracic ganglion. Staining with methylene blue demonstrated that in Homarus vulgaris and Cancer pagurus these nerves, which we term the posterior stomach nerves (p.s.n.), contain numerous cell bodies.

The morphology of the cell bodies in the p.s.n. strongly resembles that of previously described crustacean mechanoreceptors (for example, Pabst & Kennedy, 1967) and suggests that the p.s.n. have a sensory function. The position of the nerve endings on the posterior arch of the gastric mill suggests that if the nerves have a proprioceptive function the information transmitted centrally affects the output from the stomatogastric ganglion to the muscles of the mill.

The present work demonstrates the validity of these assumptions. The p.s.n. does contain neurones which respond to normal movements of the gut, and stimulation of a p.s.n. when it is isolated peripherally but connected centrally does affect the output from the stomatogastric ganglion. This section describes the anatomy and physiology of the p.s.n. sensory system in some detail.

2.1.2. Materials and Methods

Homarus vulgaris and Cancer pagurus were obtained locally. Specimens of Palinurus vulgaris were obtained from W. Harvey, Fish Merchant, Penzance, Cornwall. The Homarus used were between 20 and 22 cm long and the Cancer between 15 and 17 cm broad across the carapace.

The methods of Alexandrowicz and Whitear (1957) were used for the methylene blue staining. In both the lobsters and crabs the gastric mill is covered with thick white tissue. This tissue had to be removed gradually as the nerves stained to facilitate further staining. For the examination of the nerve with the electron microscope the tissue was fixed in 2 per cent osmium tetroxide solution diluted with an equal volume of sea water. Acetone was used as the dehydrating agent and Araldite as the embedding medium. Thick sections were cut with a Porter-Blum microtome and stained with toluidine blue. Thin sections

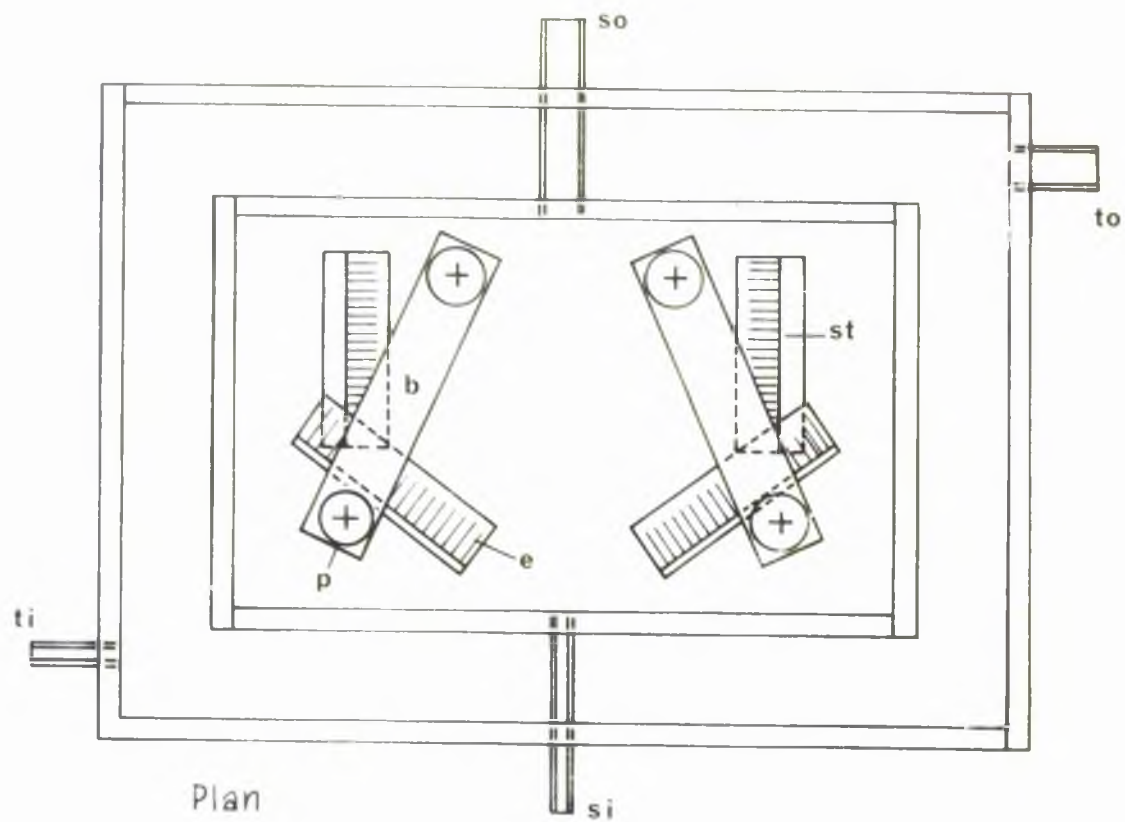
were cut with an LKB microtome, stained with lead citrate and uranyl acetate, and examined with an AEI EM 6B electron microscope.

For the electrophysiological experiments two preparations were used. After induced autotomy of the chelae and walking legs Cancer provided a minimally dissected preparation by simple removal of the carapace and epidermis over the gastric mill. The stomach was always cleared several times by means of a hypodermic syringe. Although the p.s.n.s. were always near the exposed surface it was usually necessary to isolate the nerves from the surrounding tissues. In order that the p.s.n.s. could be located easily and a reasonable length of the nerves exposed, the large specimens were used. This produced difficulties when the chelae were removed because it was not always possible to make the animal autotomize these limbs. If these limbs were removed surgically the blood losses were stemmed with tissue paper. After dissection the animal was transferred as quickly as possible to an experimental bath (figure 2.1.1.) containing aerated saline (Pantin, 1962). The saline temperature was kept between 7 and 12°C by a flow of water around the experimental bath. The anterior aorta was left intact in all experiments.

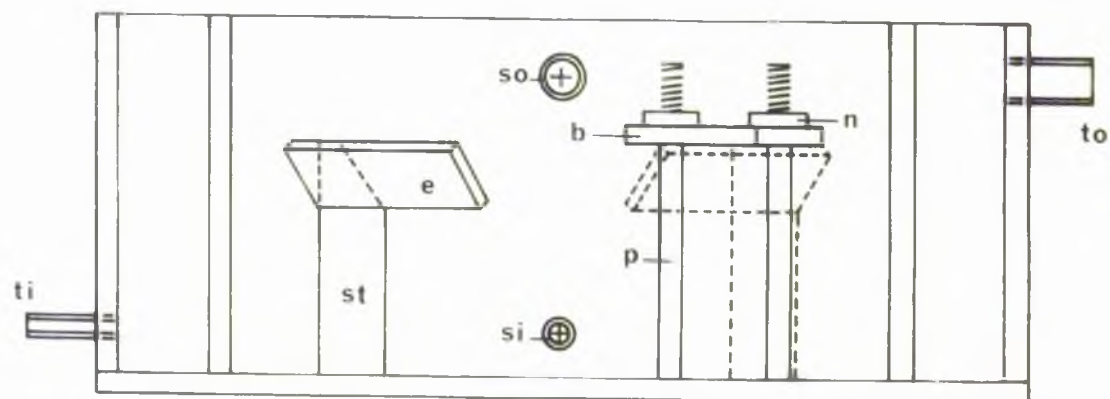
For experiments with Homarus the thorax was isolated and the digestive gland removed from it. The thorax and stomach were

FIGURE 2.1.1.

Experimental bath used to hold Cancer pagurus. The outer section was filled with sea water from the main supply. The inlet (ti) and outlet (to) allowed a flow of water to be used, this effectively kept the animal at the sea water temperature. A flow of aerated saline was also obtained in the inner bath by means of the inlet (si) and outlet (so). The animal was placed, ventral side down, on the two shaped stands (st). The forward extensions of these stands (e) held the curved anterior thorax steady. The animal was secured by two brackets (b) pushed onto pillars (p) and locked into place by the nuts (n) which were screwed tightly down.



Plan



Front Elevation

5cms.

thoroughly washed with sea water at this stage. Then the thorax was split longitudinally from the ventral surface. On one side the whole urocardiac tooth of the gastric mill was retained. This half thorax, which contained the majority of the gastric mill, was laid on its outer side and the ventral part of the stomach was removed. The apodeme of the posterior adductor muscle was then cut and the p.s.n. was separated from neighbouring tissues thus exposing a long length from the edge of the mandible to the posterior adductor muscle of the mandible. Finally the ventral part of the body wall was cut away and the apodeme of the posterior adductor muscle planed aside. This procedure enabled recording from the region of the p.s.n. proximal to the main group of cells. Recording from the distal portion of the nerve was accomplished after the entire posterior adductor muscle was removed. Cool (10 to 15°C) filtered sea water was used as the bathing fluid.

Extracellular recording and stimulation were carried out with fine wire electrodes on which the nerve was lifted into liquid paraffin. Stimulation techniques are described in the next section (2.2.2). Glass microelectrodes filled with 3M KCl and having a resistance of 20 to 30 MΩ were used for the intracellular recordings. Conventional methods of amplification and display were used.

2.1.3. Anatomy

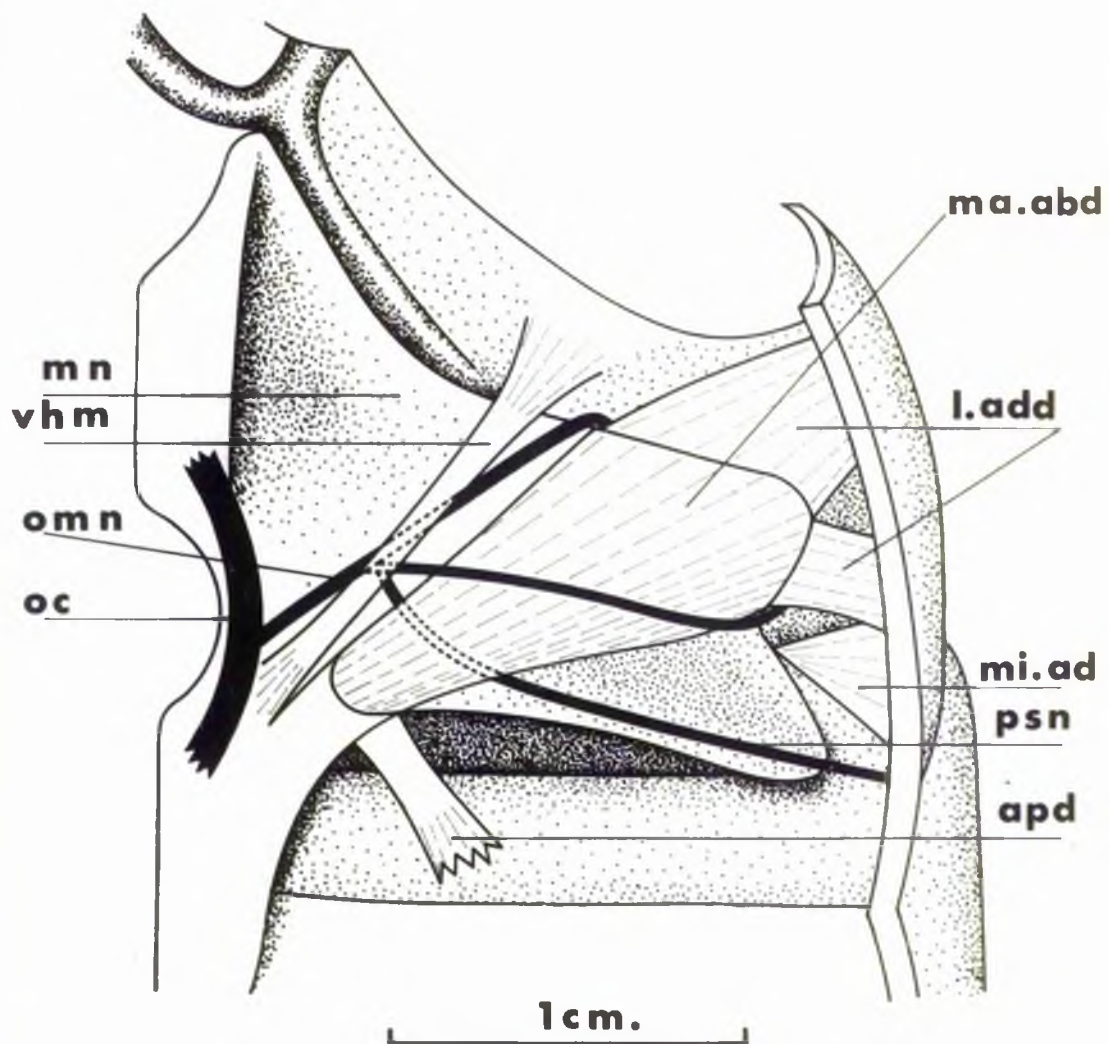
1. Homarus vulgaris

The p.s.n.s. which in Homarus are branches of the outer mandibular nerves, are symmetrically arranged on each side of the animal. Each p.s.n. proceeds ventro-laterally along the mandible and then turns dorsally to run up the side wall of the thorax just anterior to the cervical groove. The nerve then enters the ventral surface of the posterior adductor muscle of the mandible, and after passing through this muscle, runs onto the gut by way of the posterior external gastric muscle. The nomenclature used is that of Keim (1915) and Schmidt (1915).

Figure 2.1.2. shows the nerves which occur in the region of the mandible on the right side of the animal. The outer mandibular nerve leaves the circumoesophageal connective as a single trunk in the majority of animals and divides into two near the ventral head muscle. Both branches pass under the ventral head muscle and run out towards the lateral adductor muscles. Just after the first division of the main nerve the posterior branch divides again giving rise to a nerve which courses ventrally under the major abductor muscle onto the inner surface of the mandible. This is the posterior stomach nerve. The nerve runs along the posterior edge of the mandible and then onto the

FIGURE 2.1.2.

The origin of the right p.e.n. from the outer mandibular nerve in Homarus vulgaris. The dorsal part of the animal has been removed and the interior of the mandible is seen from above. The anterior of the animal is at the top of the diagram. The outer mandibular nerve (omn) leaves the connective (oc) as a single trunk. Just after the first division of the nerve the posterior branch divides again giving rise to the posterior stomach nerve (pan) which drops down onto the mandible (mn) under the major abductor muscle (ma.abd). The divisions of the outer mandibular nerve usually occur near the ventral head muscle (vhm). The other two branches of the outer mandibular nerve run out towards the lateral adductor (l.add) muscles. The posterior stomach nerve crosses the edge of the mandible behind the minor abductor muscle (ml.ad) and runs out towards the thoracic base of the posterior adductor muscle of the mandible, the mandibular apodeme (apd) of this muscle is shown here.

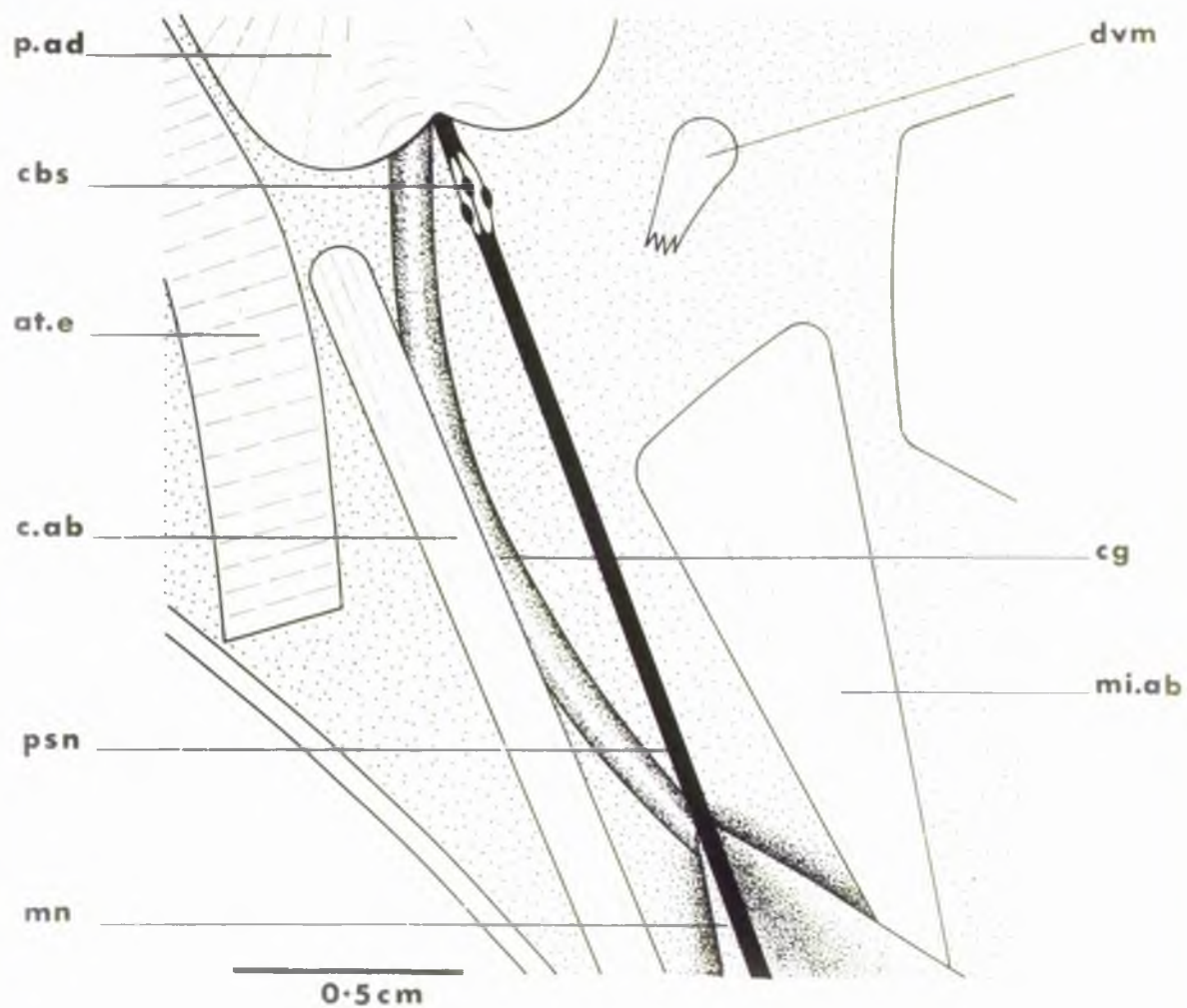


side wall of the thorax. It gives off small branches to sense organs on the surface and borders of the mandible. Many of the fibres in these branches are recurrent and run parallel to the p.s.n. for a short distance. A few cell bodies, usually with processes innervating the epidermis, are often present in the nerve trunk in this region. Most of these cells are bipolars but some tripolar cells may be seen.

Figure 2.1.3. shows the direction taken by the left p.s.n. on the lateral wall of the thorax. The p.s.n. runs in a straight line usually just anterior to the bow of the cervical groove. The nerve is usually about 250 μm in diameter in this region. The position of the individual cell bodies and the distribution of the minor branches of the nerve which innervate the epidermis vary widely even between the two sides of the same animal. The main group of cells, however, always occurs immediately before the nerve enters the adductor muscle, and the distal processes of most of these cells run to the gastric mill. It was difficult to obtain good methylene blue staining of all the cells in any one preparation. Nevertheless, in many preparations 60 cells were clearly visible and up to 80 were seen in some preparations. This compares with a total of about 105 to 125 fibres in the nerve just distal to the group of cells (see later, figure 2.1.5b.). No other cell bodies are present between the major group and the ending of the nerve on the

FIGURE 2.1.3.

The course of the left p.s.n. on the lateral wall of the thorax of Homarus vulgaris. The anterior of the animal is to the right and the top of the diagram is dorsal. The apodeme of the posterior adductor muscle of the mandible has been cut and pulled dorsally. The posterior stomach nerve (psn) crosses the mandible (mn) between the minor abductor muscle of the mandible (mi.ab) and the abductor muscle of the coxopodite of the 1st maxilla.(c.ab). It crosses the thorax in front of the bow of the cervical groove (cg). The main group of cell bodies (cbs) occurs just before the nerve enters the posterior adductor muscle (p.ad.) and about level with the thoracic base of the anterior dorso-ventral muscle (dvm). The other muscle in this region is the attractor muscle of the epimeral plate (at.e).



gastric mill but a few cells are always present between the major collection and the mandible. The cell bodies are of two types. The majority are simple bipolars of about $60 \times 40 \mu\text{m}$ (figure 2.1.4a.). The others are varieties of bipolars which tend towards a monopolar type (figure 2.1.4b.), and are of the same size as the simple bipolars. No anatomical connexions were observed between the cells in the group.

A large number of nerve fibres ramify in the thoracic epidermis surrounding the p.s.n., particularly near the base of the anterior dorso-ventral muscle. Many of the fibres run into the region from other nerves, but the p.s.n. also sends fibres to this area between the mandible and the adductor muscle. Sometimes the fibres are given off in groups which form minor branches of the nerve, and occasionally processes lead directly from a cell body in the p.s.n. out into the epidermis. The fibres innervating the epidermis often split up with characteristic multiple bifurcations which spread the distal processes from one cell over a wide area. Correlated with the branching of the main p.s.n. is a decrease in the number of fibres in it (as shown by electron microscopy) from about 160 to 180 on the thoracic edge of the mandible to about 105 to 125 immediately distal to the main group of cells (figures 2.1.5a,b). The fibres in the nerve form a fairly homogeneous collection with regard to diameter and none are larger

FIGURE 2.1.4.

Nerve cells in the p.s.n. of Homarus vulgaris, stained with methylene blue and fixed in ammonium molybdate. A. A collection of bipolar cells from the main group of cells of a preparation. The deeper-lying cells are only lightly stained but this is not due to them having thick sheaths as in the cells of Cancer (figures 2.1.9. and 2.1.10). B. A cell which is almost of a monopolar form. In both A and B the peripheral processes are at the top of the picture.

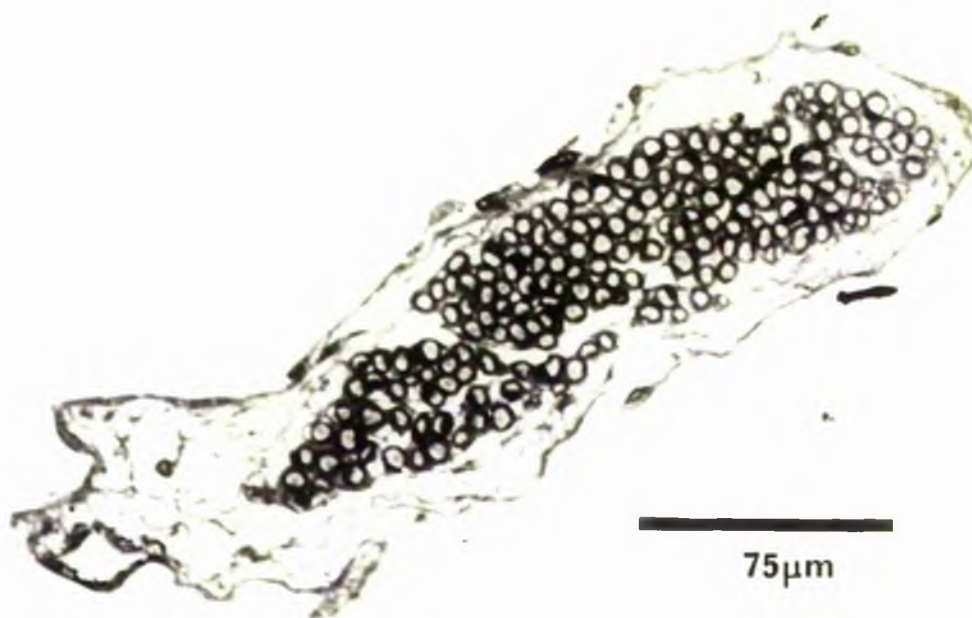


Eden Grove



FIGURE 2.1.5.

Transverse sections of the p.s.n. of Homarus vulgaris. These nerves were fixed in osmium tetroxide and stained in toluidine blue. It is sometimes difficult to decide if small objects are fibres, but electron micrographs show that there are very few small unsheathed fibres in the p.s.n. The figures quoted in the text are taken from counts of five preparations. A. Section of a p.s.n. on the thoracic edge of the mandible. There are about 170 processes in this section. B. Section of the same nerve as in A immediately distal to the position of the main group of cells. There are about 120 processes in this section.



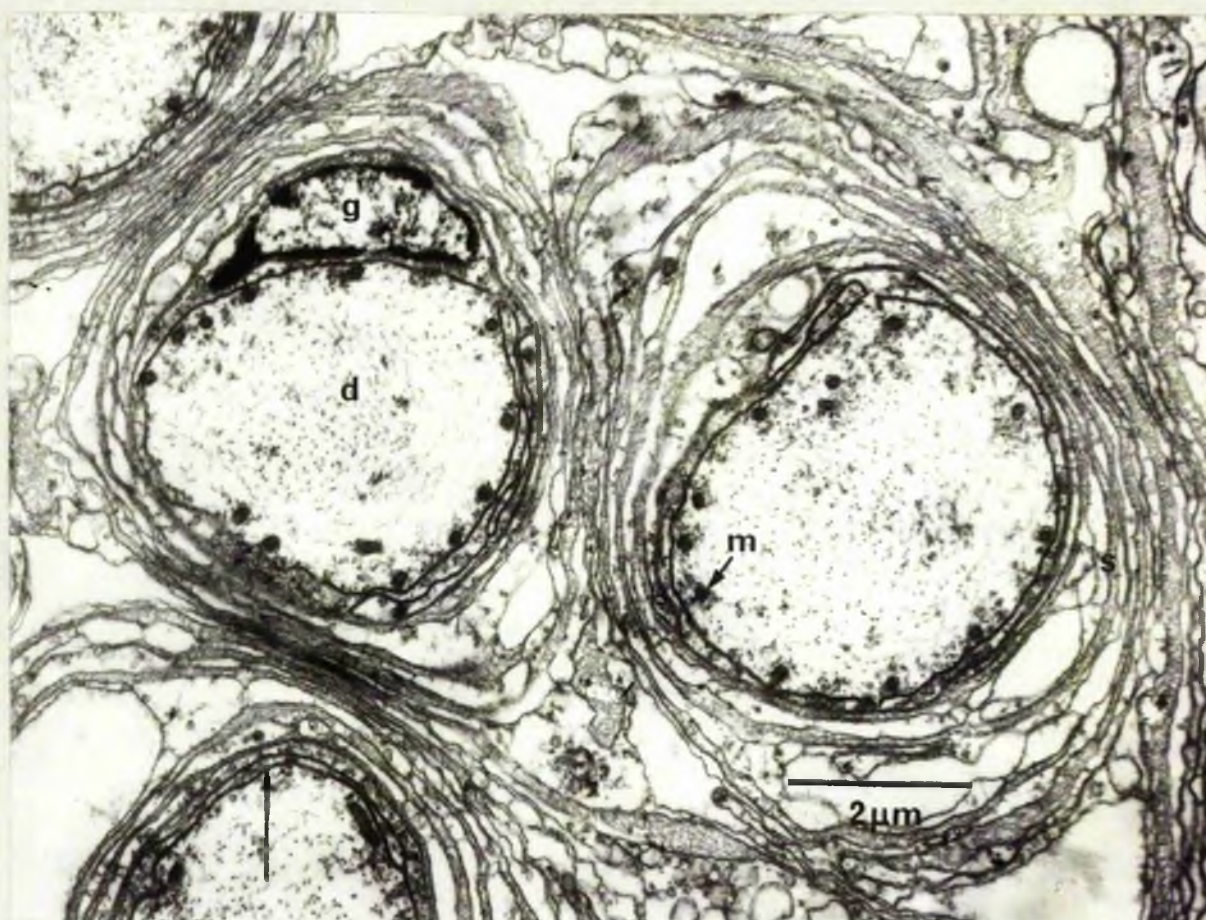
than 8µm. Near the main group of cell bodies the proximal processes are nearly all alike in structure and similar to previous descriptions of crustacean nerve fibres (for example, that of the crab leg nerve, Horridge and Chapman, 1964). One striking point of difference shown in the cross-sections of the p.s.n. is the roundness of the fibres (figure 2.1.6). There is no great difference between the nerve processes proximal and distal to the major group of cells.

The part of the p.s.n. which runs to the gastric mill is often divided as it enters the adductor muscle but these separate trunks join up within the muscle and the total diameter of the nerve decreases. Few small side branches leave the main trunks after they enter the posterior adductor muscle. Part of the mandibular nerve and a large blood vessel run in the same gap in the muscle as the p.s.n., but the p.s.n. is most superficially positioned. As the p.s.n. reaches the base of the gastric muscle it is often crossed by another nerve which supplies the body wall epidermis in this region. On the posterior external gastric muscle the p.s.n. spreads out and many of the fibres divide by multiple bifurcations as the nerve approaches the gastric mill.

The exact location of the endings of the p.s.n. on the gastric mill was difficult to ascertain. This difficulty was caused by the presence of an anastomosis between the p.s.n. and several other nerves near

FIGURE 2.1.6.

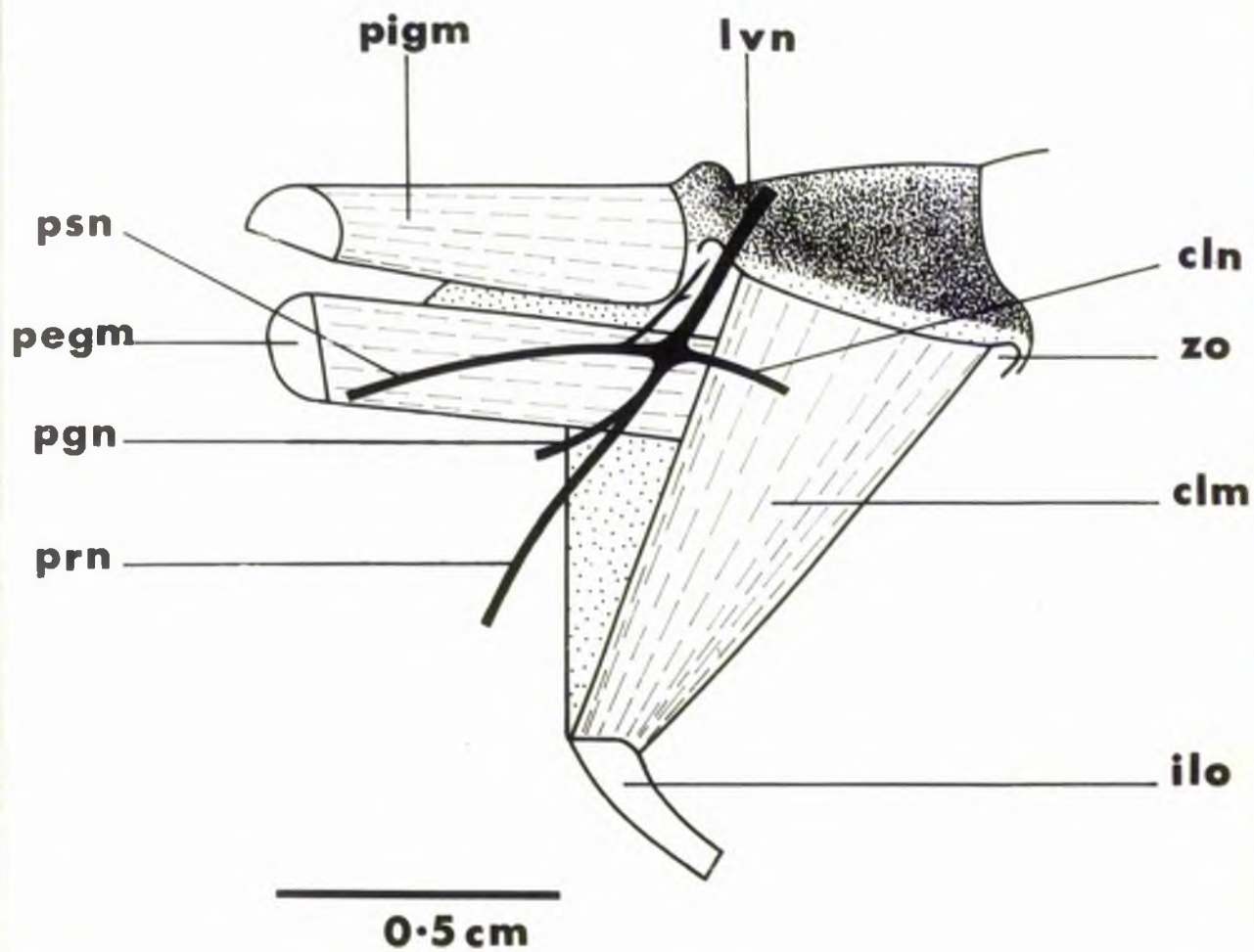
An electron micrograph of typical fibres in the p.s.n. of Homarus vulgaris. This section is of processes just distal to the main group of cells from the same nerve as is shown in figure 2.1.5. d = nerve fibre, probably the peripheral process of a cell in the p.s.n.; i = inner sheath of nerve fibre; m = mitochondrion; s = outer sheath of nerve fibre; g = sheath cell nucleus.



the gastric mill. The other nerves forming the junction are the lateral ventricular nerve, and the three nerves that run respectively over the cardio-lateral muscle, round the posterior gastric muscles, and onto the pyloric region of the gut. The lateral ventricular nerve contributes many fibres to the last two nerves mentioned. These fibres are easily traced because of their large size. Figure 2.1.7. is a diagram of this region though the precise anatomy is variable between different preparations. Often there are anastomoses, involving small numbers of fibres, between the p.s.n. and branches of the nerve running around the gastric muscles before the major junction. The p.s.n. can contribute fibres to all other nerves partaking in the anastomosis and branches of single fibres do pass into different nerves. Despite this complexity one significant feature is often seen in preparations in which the p.s.n. divides before the major anastomosis. In such preparations some branches of the p.s.n. do run to the gastric mill without encountering any other nerves (that is to say, bypassing the junction). The fibres in these branches ramify in the connective tissue which occurs in the regions between the posterior gastric muscles and the dorsal edges of the exopyloric and zygo-cardiac ossicles. (This connective tissue fills all the spaces between the ossicles of the mill and is easily distinguished from the thick white tissue which overlays the

FIGURE 2.1.7.

The peripheral termination of the right p.s.n. on the posterior of the gastric mill of Homarus vulgaris. The anterior of the animal is to the right. The lateral ventricular nerve (lvn) has been cut and pulled clear of the thick white tissue which still overlays the dorsal surface of the gut. This tissue has been removed from the base of the posterior external gastric muscle (pegm). The posterior stomach nerve (psn) runs along the ventral surface of the posterior external gastric muscle and gives off a small branch to the connective tissue which invests the region between the posterior gastric muscles (pigm - posterior internal gastric muscle) and zygocardiac ossicle (zo). The posterior stomach nerve then anastomoses with the lateral ventricular nerve. From this anastomosis a nerve (cln) runs over the cardiolateral muscle (clm). The thick white tissue has been removed from this muscle down to the infero-lateral ossicle (ilo). Other nerves from the anastomosis run over the pyloric region of the gut (prn) and around the gastric muscles (pgn).



gastric mill.). Nerve fibres also run onto the top of the mill between the insertion of the posterior gastric muscles and the propyloric ossicle, and laterally along the dorsal edge of the zygo-cardiac ossicle. In all preparations there were nerve fibres in these regions and often some could be traced back to the p.s.n. Though some fibres run deeply into the connective tissue none were traced directly onto the ossicles.

ii. Palinurus vulgaris

In this species the p.s.n. follows a similar course to that described for Homarus. The main group of cell bodies is usually nearer the mandible than in Homarus, and its position is often obscured by a blood vessel. The cell bodies are large (80x40µm) and are generally pear-shaped with the blunt end facing centrally. There is also a small proportion of cells tending towards the monopolar type. The p.s.n. is commonly divided into a number of trunks distal to the main group of cells. The nerve runs external to the posterior adductor muscle of the mandible and through a gap in the anterior dorso-ventral muscle.

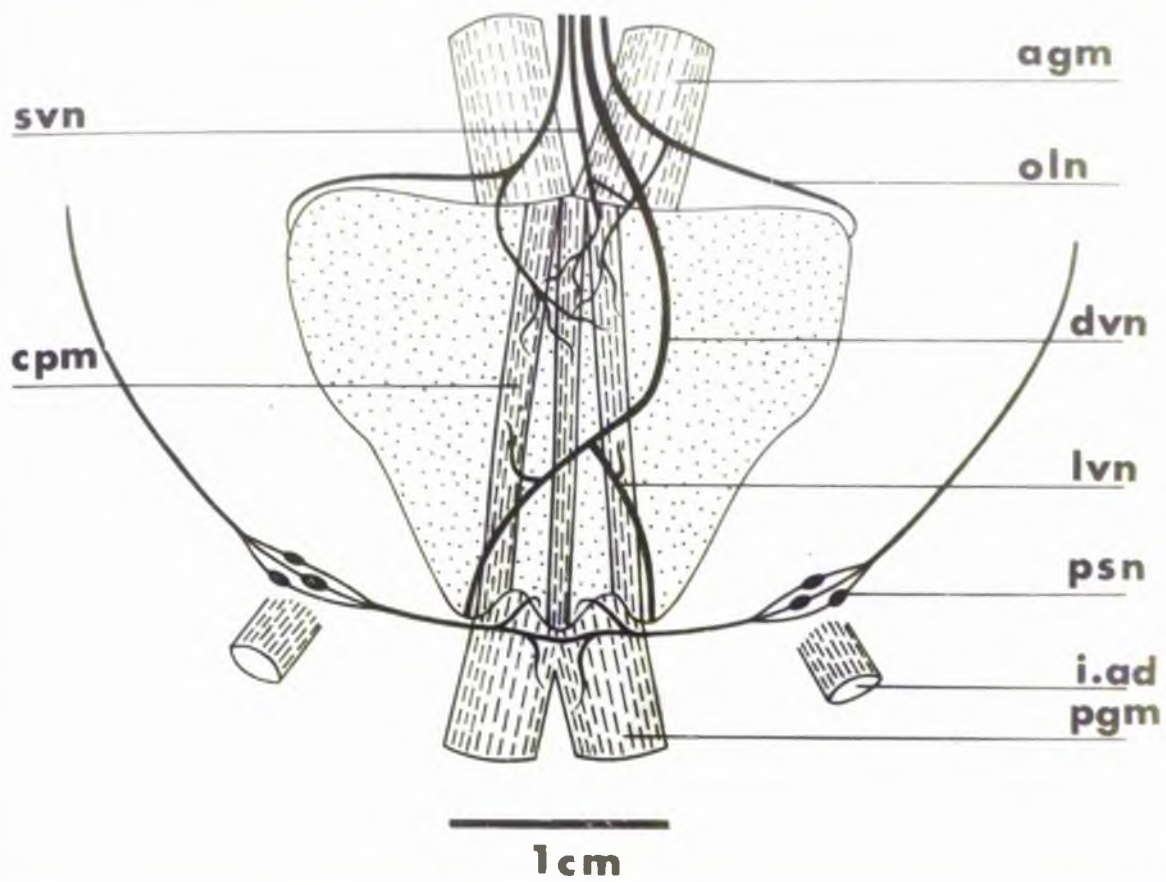
I did not continue working with this species because yellow tissue (see Alexandrowicz and Whitear, 1957) obscured the central end of the p.s.n., and the gastric mill is much weaker than that of Homarus.

iii. Cancer negurus

The course of the two p.s.ns. on the dorsal surface of Cancer (figure 2.1.8.) is similar to that described for Pugettia by Heath. At the base of the posterior external gastric muscles each nerve runs laterally for a short distance and lies alongside a small muscle which is an extension of the internal abductor muscle of the mandible. This small extension is not described by Pearson (1908) in his monograph on Cancer. The p.s.n. often divides in order to pass round this muscle. Most of the cell bodies are in a group which is near this muscle or between it and the gut. (In a few preparations the group of cells was at some distance central to the internal abductor). From the small muscle the p.s.n. passes forward and becomes associated with the epidermis before reaching the cuticular insertion of the external abductor muscle of the mandible. A few cell bodies are often found along this region of the nerve between the two muscles. The nerve runs centrally along the external abductor muscle and then into the thoracic ganglia with the mandibular nerve. A group of 10 to 15 cell bodies occurs in the common root of the p.s.n. and the mandibular nerve. Some of the distal processes from these cells innervate the tissue overlaying the mandible but many appear to run in the p.s.n. There are usually 20 to 25 cells which stain in the main group of cells.

FIGURE 2.1.8.

The arrangement of the stomatogastric nervous system on the dorsal surface of the gastric mill of Cancer pagurus. The thick white tissue has been removed from the dorsal surface of the gastric mill and the p.e.n.s. cleared of obscuring viscera up to the point where they descend ventrally. On each side of the mill the posterior stomach nerve (psn) contains a group of cell bodies when it is near the internal abductor muscle (i.ad). The p.e.n.s. then run onto the gut near the insertion of the posterior gastric muscles. For clarity the usual anastomosis with the lateral ventricular nerves (lvn) is omitted. The p.e.n.s. join up behind the propyloric ossicle and fibres from one side probably often cross to the other. The region in front of the propyloric ossicle is heavily innervated. The connective tissue here overlays the reflected urocardiac ossicle (see figure 2.1.11). Anteriorly there are four nerves arising from the region of the stomatogastric ganglion. The largest nerve is probably analogous to the dorsal ventricular nerve (dvn) of Homarus minus the innervation of the cardio-pyloric muscles (cpm) which here are innervated by a separate short ventricular nerve (svn). This is often a branch of the d.v.n. close to the ganglion. The dorsal ventricular nerve divides into two lateral ventricular nerves. The paired outer lateral nerves (oln) are probably analogous to the median ventricular nerves of Homarus as they appear to innervate the same muscles. There are variable anastomoses between these four stomatogastric nerves. The stomatogastric ganglion lies on the anterior surface of the cardiac stomach and it is not shown in this diagram. agm, anterior gastric muscles; pgm, posterior gastric muscles.



Cross-sections of the nerve show that it has a much thicker sheath than in Homarus and that many of the cell bodies also have thick individual sheaths (figure 2.1.9.) All the p.s.n. cells are bipolars with dimensions of between $30 \times 20 \mu\text{m}$ and $50 \times 30 \mu\text{m}$. Good staining with methylene blue was difficult to achieve. Many of the cells appear to have a large pale surround (figure 2.1.10.) The sheaths probably explain the capricious results of the methylene blue staining. The cells with the thick sheaths are of the same sizes as the other cells in the nerve which stain normally. However, sequential transverse sections of the nerve confirm an impression gained from methylene blue staining, that the initial parts of the peripheral processes of many cells are of greater diameter than the central processes.

In the region of the nerve between the two mandibular muscles (i.e. central to the usual position of the cell bodies) there are usually 40 to 45 fibres. These fibres are similar in structure to those described for Homarus. The counts of fibres and cell bodies in the nerve are again fairly close. As they approach the gut many of the fibres in the p.s.n. divide into smaller processes, again with the typical bifurcations.

The p.s.n. on each side runs on to the gut near the base of the posterior external gastric muscles. Each nerve usually anastomoses

FIGURE 2.1.2.

Transverse section of part of the p.s.n. of Cancer pagurus. This nerve was fixed in osmium tetroxide and stained with toluidine blue. ls = individual sheath of a cell body; cb = cell body; ns = sheath of p.s.n. The large peripheral processes (p) of some cells are at the top of the picture.

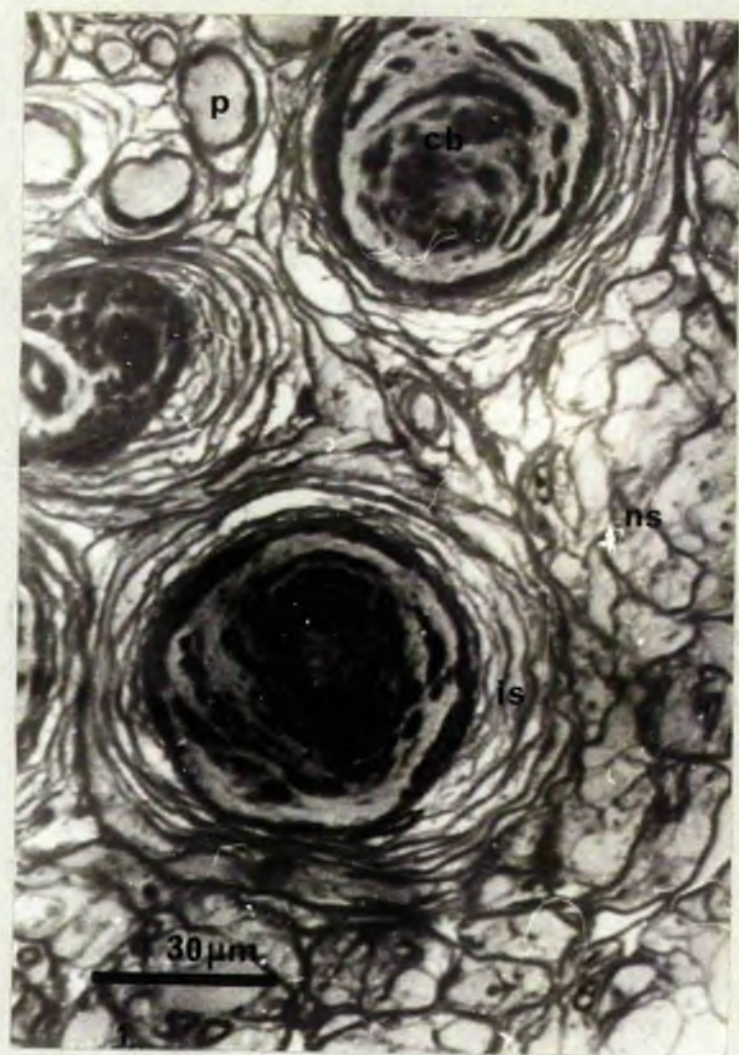
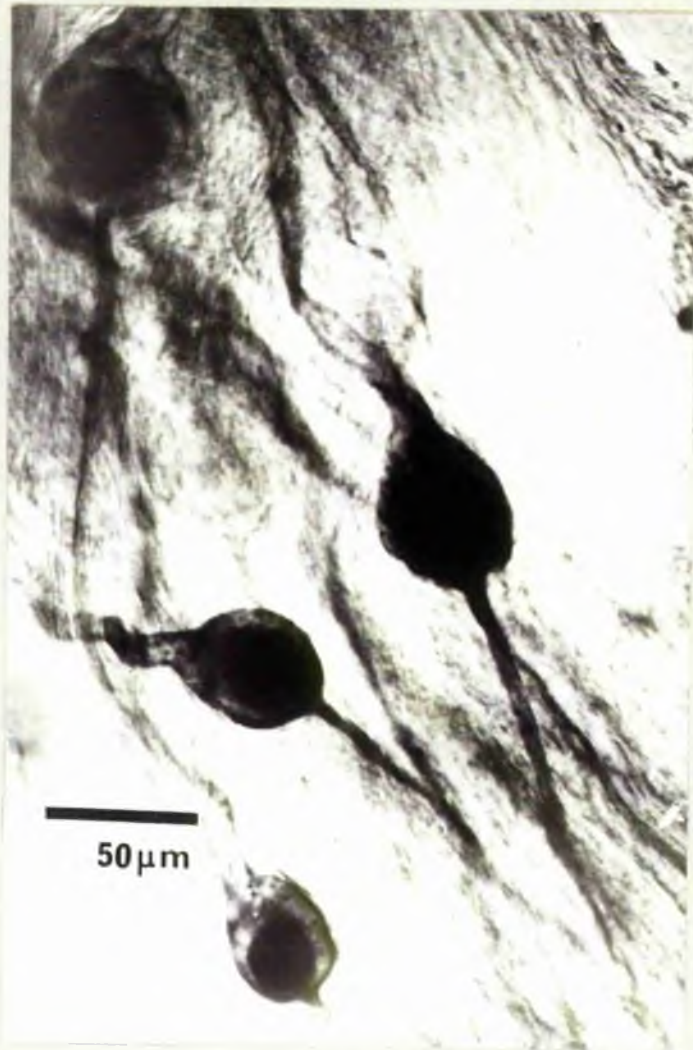


FIGURE 2.1.10

Nerve cells in the p.s.n. of Cancer sagurus. These cells were stained with methylene blue and fixed in ammonium molybdate. The large diameter peripheral processes of the cells run towards the top of the picture. These cells show the clear halo around the stained part of the neurone. This effect is almost certainly caused by the failure of the large individual sheaths of the cells (figure 2.1.9.) to stain.



with the lateral ventricular nerve on the same side of the gut and then joins the other p.s.n. just behind the propyloric ossicle. Branches of the posterior stomach nerves ramify in the connective tissue which invests the ossicles of the mill but the innervation appears to be concentrated more around the propyloric ossicle than in Homarus.

In Carcinus maenas there is a p.s.n. which runs on to the gastric mill in a similar manner to that described for Cancer pagurus. This nerve also contains a group of cells in the same position as in Cancer. Galathea strigosa also possesses a nerve running on to the posterior part of the gut in the same position as in Cancer. It was difficult to identify cell bodies in Galathea because in the small number of specimens examined the nerve joined the epidermis much closer to the gut than in Cancer and made vital staining difficult.

2.1.4. Physiology.

I. Cancer pagurus

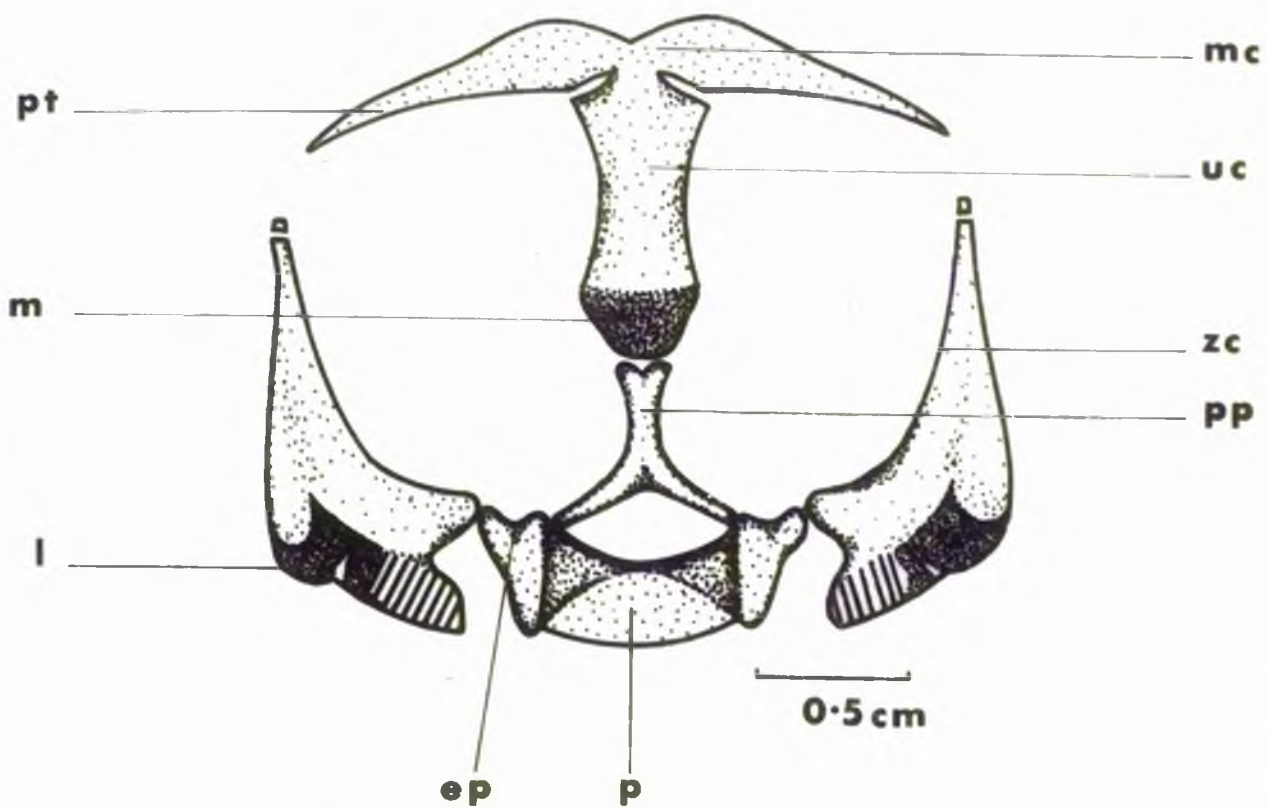
This species was used for most of the work as it was available in large numbers and because the nervous system on the dorsal surface of the mill is more accessible than that of Homarus. Figure 2.1.8. shows the normal resting position of the gastric mill and the anatomy of the nerves running posteriorly from the stomatogastric ganglion in Cancer. This diagram should be compared with Maynard's (1966)

figure for Homarus. In Cancer the p.s.n.s. can be isolated and physiological activity recorded with little difficulty. The nerves in Homarus are overlain and obscured by the posterior external gastric muscles. The disadvantage of using Cancer is that the stomatogastric ganglion usually lies on the anterior surface of the gut almost at right angles to the plane of the nerves shown in figure 2.1.8. The relevant ossicles of the gastric mill of Cancer are illustrated in figure 2.1.11. The anterior gastric muscles insert on the medial part of the pterocardiac ossicles. The posterior gastric muscles insert on the pyloric and exopyloric ossicles, and the cardiopyloric muscles span the gap between the anterior and posterior arches of the mill. In open preparations the movements of the major dorsal ossicles can be followed during normal and induced activity.

I was not able to make a detailed study of the natural movements of the gut since in most preparations the gastric mill did not move spontaneously. In those preparations in which the mill did move after the carapace was opened the movements did not last longer than a few minutes. Starvation of the animals or starvation followed by feeding just before the operation, had little effect on the number of animals in which the mill moved spontaneously after dissection. The movements of the active mills consist of a synchronous motion of the

FIGURE 2.1.11.

The dorsal ossicles of the gastric mill of Cancer pagurus as seen from the inside of the mill. The anterior arch consists of the median mesocardiac ossicle (mc) and on each side a pterocardiac ossicle (pt). The distinction between these and the median urocardiac ossicle (uc) which bears the medial tooth (m) is emphasized in this drawing. Seen from this aspect the medial tooth would lie above the propyloric ossicle (pp) in life. Here the propyloric ossicle is shown in a more posterior position and displaced more than 90° from its usual alignment. The apex of this ossicle connects with the urocardiac ossicle, and the base with the posterior arch. The medial tooth is reflected backwards at rest. The medial pyloric ossicle (p), two paired exopyloric ossicles (ep), and the two zygocardiac ossicles (zc) make up the posterior arch. The zygocardiac ossicles connect with the outer ends of the two pterocardiac ossicles in life. The zygocardiac ossicles each bear a lateral tooth (l). In life these teeth would be rotated in towards the medial tooth and would lie above it if seen from this aspect.



mesocardiac ossicle backwards and a smaller forward motion of the propyloric ossicle. In mills 1.5 cm long from the mesocardiac to the propyloric ossicle, the mesocardiac ossicle was often observed to move backwards by 0.4 to 0.5 cm. These movements were followed by a slower concurrent return of the ossicles to their resting positions. The time taken for the complete cycle of movements to occur varied from 2 to 5 s. It was occasionally possible to observe one of the ossicles moving without the other also moving, but normally the two movements were co-ordinated. The movement of the mesocardiac ossicle necessarily imposes a movement on the central parts of the pterocardiac ossicles, and of the urocardiac ossicle. The movements of the propyloric ossicle probably require simultaneous movements of all the other ossicles in the posterior arch of the mill. These normal movements must involve backward and forward motions of the medial tooth and probably movements of the lateral teeth. In a small number of preparations the mesocardiac ossicle also moved forward from the resting position taken up between the normal cycle of movements. This movement appeared to be caused by contraction of the anterior gastric muscles and not just relaxation of the cardiac-pyloric muscles.

In about half of the preparations examined movements of the gastric

mill could be evoked when the d.v.n. (dorsal ventricular nerve) and s.v.n. (short ventricular nerve), nerves which run over the dorsal surface of the gastric mill from the stomatogastric ganglion, were isolated from the ganglion and stimulated by a burst of electrical shocks. The resultant cycle of movement was the same as that observed in minimally dissected crabs and of the same order of magnitude. A forward motion of the mesocardiac ossicle between the cycles was never evoked by stimulation but sometimes the whole mill moved backwards. Equivalent stimulation of the peripheral part of the p.s.n., when it was isolated from the CNS., evoked no noticeable movement of the gut. This indicates that the p.s.n. probably lacks motor fibres. Concurrent stimulation of the d.v.n. and s.v.n. nerves, and the peripheral part of the p.s.n. did not interfere with the movements of the mill. This indicates that the p.s.n. does not carry inhibitory fibres.

Spontaneous electrical activity was recorded in the p.s.n. when there was no visible movement of the stomach. This activity was recordable distal and proximal to the major group of cells. It is a fairly regular discharge. If the nerve was cut central to the usual position of these cells the activity did not noticeably decrease in that part of the nerve distal to the cut. In the central stump of the nerve few units were evident after the injury discharge.

Normal movements of the gastric mill caused by stimulation of the d.v.n. and s.v.n. nerves evoked changes in the activity of units in the p.s.n. (figure 2,1,12.) The units recorded all had a phasic and a tonic component. Larger movements of the mill produced an increased response from the units. Similar units were readily recorded in the p.s.n. following simple mechanical movements of the mesocardiac ossicle which simulated normal movements (figure 2,1,13). A range of resting frequencies and responses to a similar stimulus were encountered but no pure phasic or tonic receptors were found. The units are sensitive to the rate (figure 2,1,14.) and to the magnitude of displacement. Smaller responses could be obtained from these units by forward movements of the mesocardiac ossicle. It is possible that the extent of movement necessary to evoke these responses is outside the normal physiological range of movements. Unfortunately it was difficult to stimulate all of the nerves which innervate the anterior gastric muscles in order to check the response. The single units recorded in the p.s.n.s. could be stimulated by probing widely spaced (even contralateral) parts of the posterior of the gastric mill. Great care was necessary in these experiments to prevent the p.s.n. from being stretched by movements of the mill and perhaps thereby producing false results.

The electrical activity in the nerves running over the gastric

FIGURE 2.1.12.

Cyclic movements of the gastric mill of Cancer pagurus evoked by stimulation of the nerves from the stomatogastric ganglion which run posteriorly over the dorsal surface of the mill; and the response of a unit in the left p.s.n. recorded at a position central to the main group of cells. In all records the top line shows when a stimulus is delivered. The second line is a record of the movements of the left pterocardiac ossicle midway along its length monitored with a mechano-electrical transducer (RCA 5734). The bottom line is a record of a unit in the p.s.n. A. The response to a stimulus of 10 V. B. The response to a stimulus of 15 V. The time base is the same as in A. The transducer rod on the pterocardiac ossicle moved back 2mm at this stimulus intensity. C. The response to a stimulus of 10 V with the speed of film increased to twice that in A. D. The fourth and fifth responses in a series of equal stimulations of 10 V with the same time base as in A.

A



5 secs

B



C



D

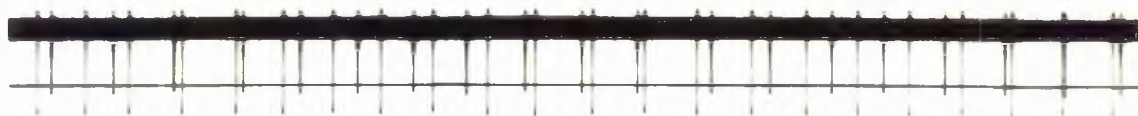


FIGURE 2.1.13

The response of two units in the left p.s.n. of Cancer pagurus to varying sizes of movements of the central part of the pterocardiac ossicle on the same side of the mid l. The movements of the gut were made by moving a probe attached to the pterocardiac ossicle. The second line on the trace shows the movement of the probe which was driven from a Servomex LF. 51 Mk II waveform generator.

A. Two units firing at constant rate with no visible gut movements.
B. The response to a backwards movement of 3mm of the pterocardiac ossicle in 0.9s. C. The response to a movement of 2.25mm of the ossicle. D. The response to a movement of 1.8mm. In the graph (over page) the response of the large unit (light line) and small unit (dark line) to this last movement is shown in an instantaneous frequency curve. This plots the reciprocal of the interval between successive spikes ($1/\Delta t$) against the midpoint between successive spikes.

A



B



C



D



5secs

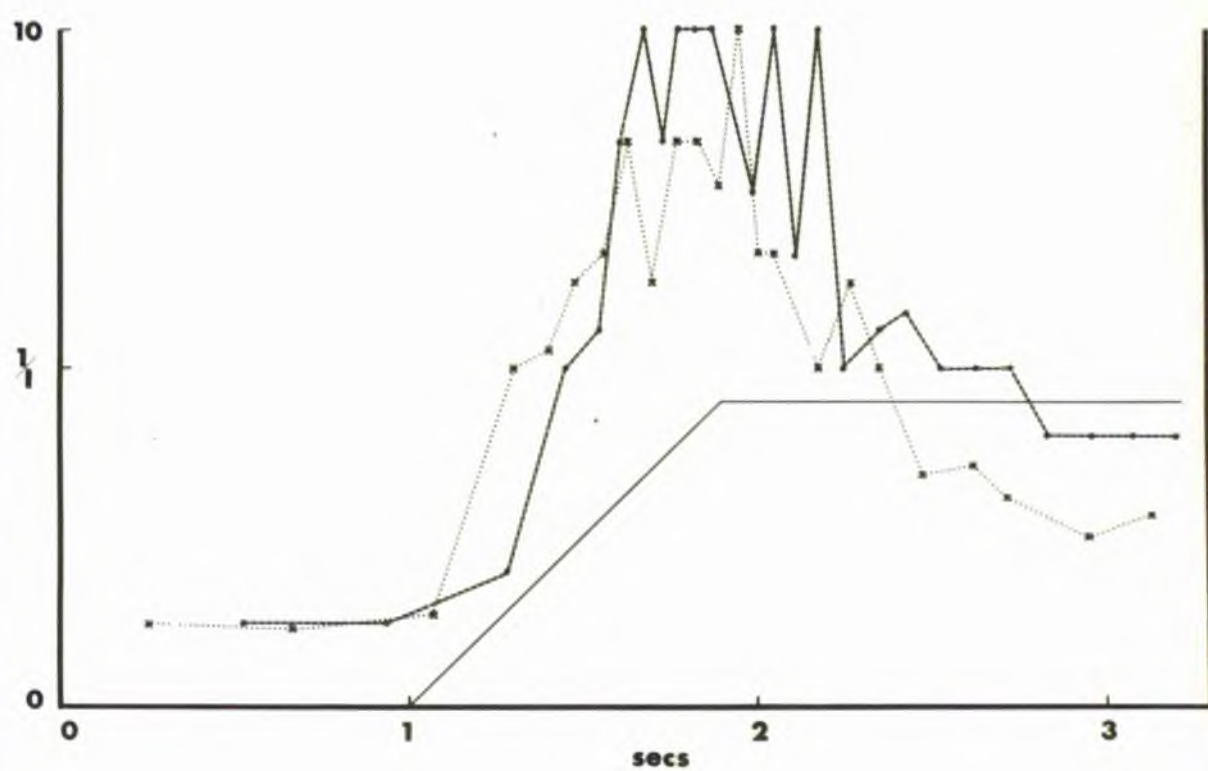
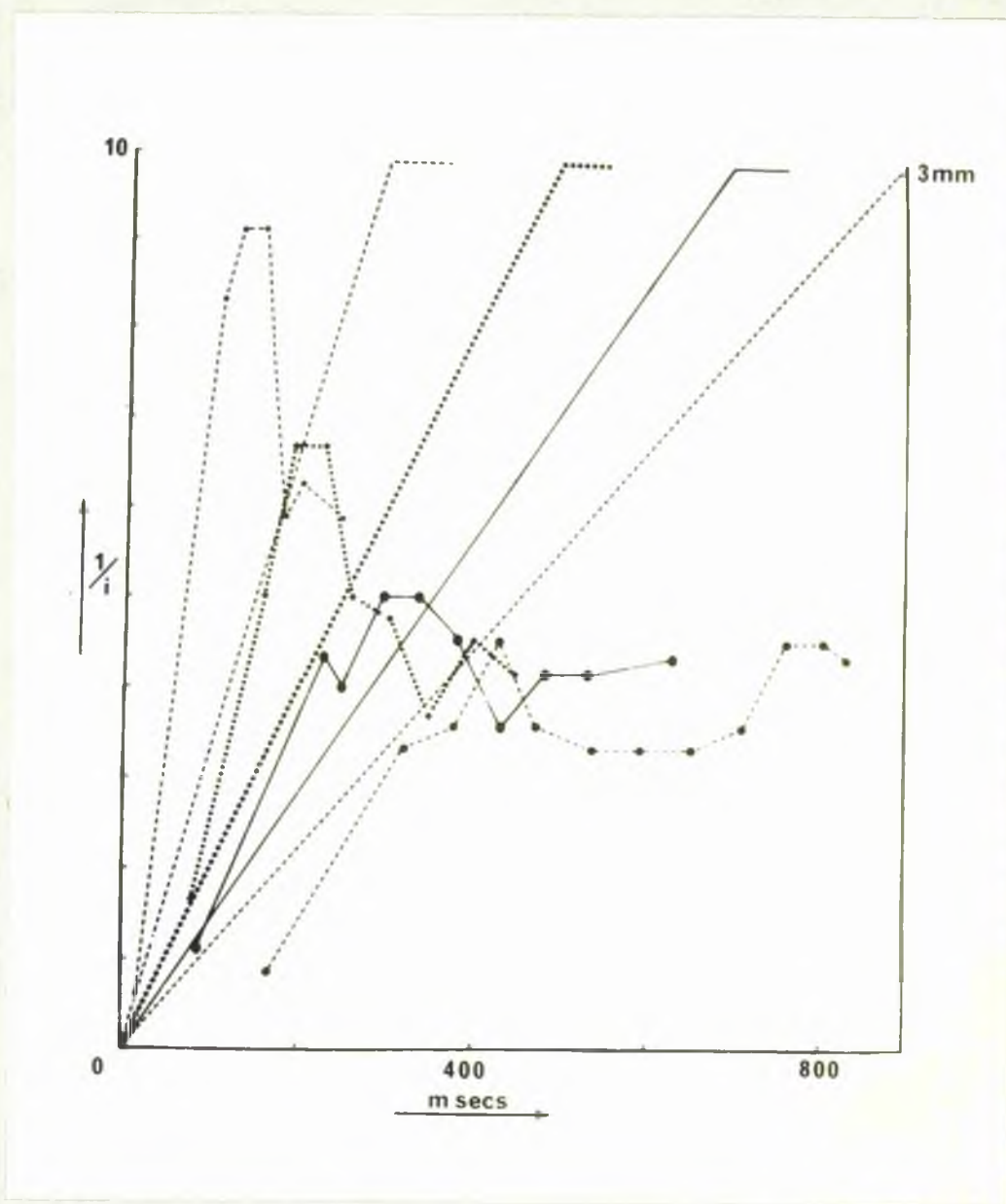


FIGURE 2.1.14.

The response of a unit in the left p.s.n. of Cancer sagurus to varying speeds for a 3mm movement of the pterocardiac ossicle on the same side of the mill. The second trace shows the movement of the mill. A. Response to the movement in 300 ms. B. Response to the movement in 500 ms. C. 700 ms. D. 900 ms. The movement was evoked by the same means as described in figure 2.1.13. In the graph (over page) the response of this unit to the four speeds of movement is shown in an instantaneous frequency curve. This curve plots the reciprocal of the interval between successive spikes against the midpoint between successive spikes. The speed of each movement and the unit's response to that movement are shown in the same type of line.





mill from the stomatogastric ganglion often occurred in regular patterned bursts (figure 2.1.15.) This patterning remained if the peripheral part of the nerve was cut away from the gut. The nerves most thoroughly studied were the paired outer lateral nerves in the group which run back over the mill (figure 2.1.8.). These nerves are almost certainly equivalent to the median ventricular nerves of Homarus as described by Maynard (1966). Recordings were usually made from these trunks after they had crossed the anterior gastric muscle. There is a good deal of variation in the anatomy of the nerves on the dorsal surface of the mill but this region of the outer lateral nerves could always be located with little difficulty. In many preparations the recordings from the nerve demonstrated the presence of two units which fired in a rhythmic manner (figure 2.1.15.) The small unit tended to cease discharging quickly, leaving the large unit alone. In about 63 per cent of the experiments performed repetitive electrical stimulation of either one of the peripherally isolated p.s.n.s. was followed by changes in the activity of the units in the outer lateral nerve. No great difference was noted between stimulation of the p.s.n. on the ipsi- or the contralateral side of the gut to the nerve under study, though the majority of the experiments were performed with the stimulation applied to the contralateral p.s.n.

Figure 2.1.16. illustrates an example in which stimulation of the

FIGURE 2.1.15.



Examples of the patterned output in nerves originating in the stomatogastric ganglion. A. Output in the d.v.n. This record was filmed at half of the film speed of record B. It demonstrates the regularity of the output over a period of time, which was obtained in good preparations. B. Top line is a record for the output in the d.v.n. Bottom line is the output in the left o.l.n. of the same preparation recorded simultaneously. The time mark applies to this trace. C. The output in the right and left l.v.n.s. of a preparation, recorded at twice the film speed of B. Most preparations had recognizably similar units in these nerves but many were much more irregular. The cycle frequency also varied in different regular preparations.

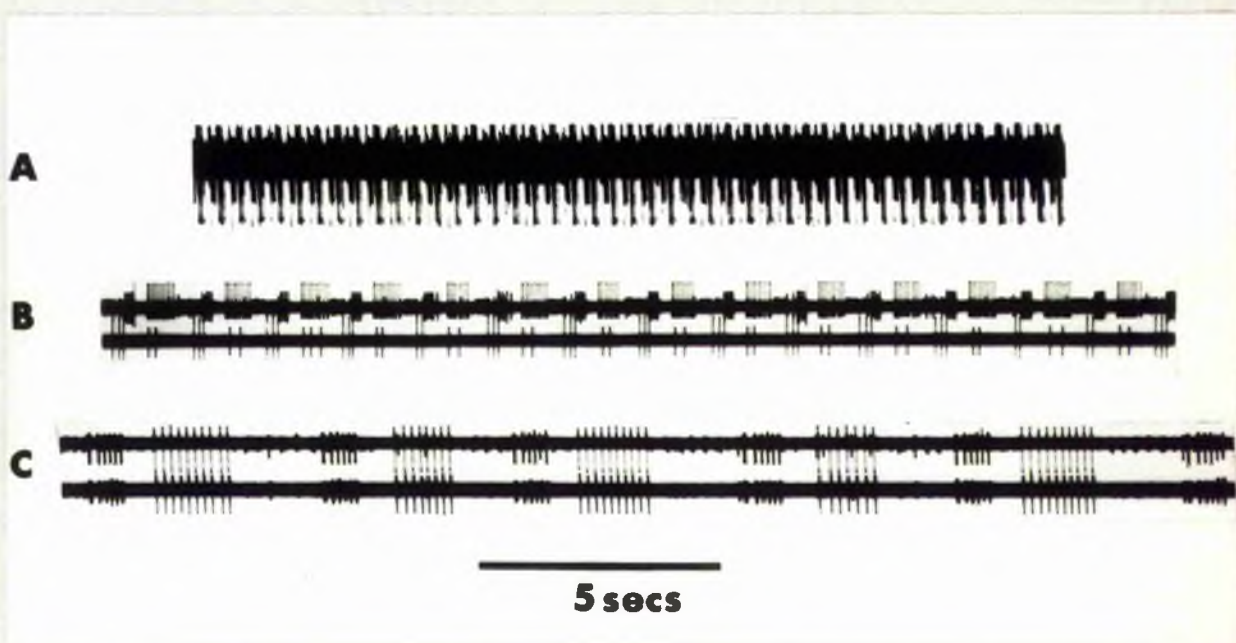
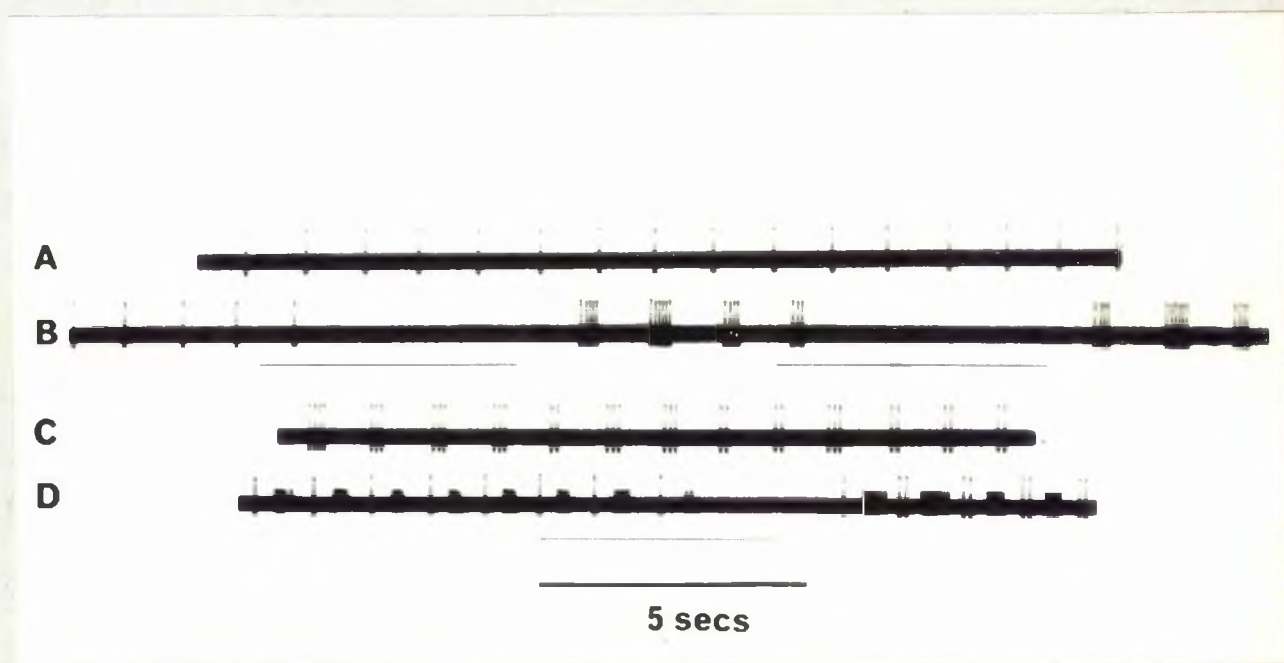


FIGURE 2.1.16.

The response of two units in the left outer lateral nerve of Cancer pagurus to repetitive electrical stimulation of the cut central end of the right p.s.n. A. Output in the peripherally isolated nerve when the small unit ceases firing. B. Two repetitive stimulations in a short series are indicated by the second line. Note inhibition and rebound. C. Record of activity after the series of stimulations. This output lasted for several minutes after which another series of stimuli were given. The 'normal' activity then returned to the nerve. D. The effect of repetitive stimulation of the p.s.n. on this activity.



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contralateral p.s.n. produced an inhibition of the firing of the large unit in the outer lateral nerve followed by an increase in activity when the stimulus ceased. In the series shown in figure 2.1.17. the inhibition and rebound become progressively greater. This unit is probably equivalent to the m unit described by Maynard (1966).

A different result sometimes obtained was a direct increase in the activity of the large unit upon p.s.n. stimulation. In a few experiments stimulation of the p.s.n. initially caused an inhibition of the unit followed by the poststimulatory increase in activity and later in the experiment only caused a direct increase in activity. This increase may have been due to the progressive deterioration of the preparation. Occasionally, however, the patterned activity of the two units returned during the stimulation experiments (figure 2.1.16.). These experiments may therefore indicate a lability of the junctional events in the interpolated stomatogastric ganglia. The experiments were controlled by stimulations applied to the viscera and the gastric mill to ensure that there was not some general effect on the animal produced by the shock of the stimulus. Such controls and experimental stimulations could be repeatedly alternated with no change in the response to the experimental stimulus, and no response to the shocks applied elsewhere. In a small number of experiments the gut responded with a normal cycle

FIGURE 2.1.17.

The response of the large unit in the left outer lateral nerve of Cancer pagurus to a series of repetitive stimulations of the contralateral p.e.n. The records follow on from each other in the order A to D. Five-second repetitive stimulations of 10 V for 1 ms at 20/s were given at five second intervals. Note the progressively increasing inhibition and rebound.

A



B



C



D



5 secs

of activity after a stimulus to the p.s.n. (figure 2.1.18).

ii. Homarus vulgaris

Spontaneous electrical activity was recorded in the p.s.n. when there were no visible gut movements. This spike activity was recordable both distal and proximal to the major group of cell bodies. Transections of the nerve central to the group of cells left the same level of activity in the distal section but very little in the central root. Sectioning the nerve distal to the cell bodies left the same level of activity in the terminal portion. This activity was recordable in the part of the p.s.n. on the posterior external gastric muscle very close to the gastric mill.

Movements of the ossicles of the mill which mimicked normal activity observed in minimally dissected animals, evoked changes in the activity of units in the p.s.n. in a similar manner to that described for Cancer. Movements of the pyloric region alone of the gut had little effect on the activity of the p.s.n. whereas movements of the teeth of the mill from their normal alignment (as might be produced by a large amount of food being in the stomach) evoked responses in units on the p.s.n. Intracellular recordings from the cells in the main group confirmed that the cells are mechanoreceptors responding to normal movements of the gastric mill (figure 2.1.19).

FIGURE 2.1.18.

Transducer records of normal and induced movements of the left pterocardiac ossicle of the gastric mill of Cancer Pagurus. A. Recording of spontaneous normal cyclic activity. B. The thick line indicates that a repetitive stimulus is given to the left p.s.n. when it is cut peripherally but connected centrally (as in figures 2.1.16. and 2.1.17.). Here the stimulus evokes a movement of a normal amplitude and length. C. A stimulus immediately after a small response. D. A stimulus in the middle of an evoked movement considerably extends the response. Upward movement of transducer records posterior movement of the ossicle.

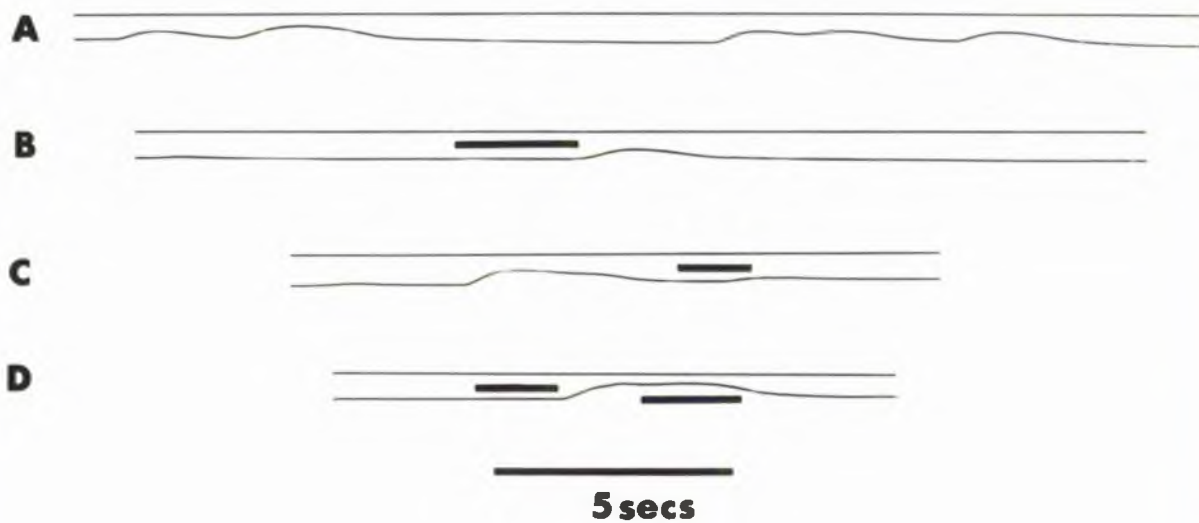


FIGURE 2.1.19.

Intracellular recordings from a cell in the p.s.n. of Homarus showing the response to forward movements of the urocardiac ossicle. A. The response to a movement of 3mm. Time mark for A to C is 1 s. B. The response to a similar movement filmed at twice the speed of A and C. This movement was slightly smaller than in A but started from in front of the resting position. C. The response to the fourth and fifth in a series of equal stimulations. D. Records of spikes filmed at higher speed to show the form of the impulse. Calibration 80 mV and 50 ms.



Section 2.2. The Oesophageal - Mandibular Receptor System

2.2.1. Introduction

The major difficulty with the investigation of the p.s.n. sensory system was that the gastric mill rarely functioned in dissected animals. Therefore the central effects of p.s.n. stimulation were difficult to analyse.

I was interested to find that the oesophagus and labrum of Homarus vulgaris continue to be mobile for a prolonged period after severe dissection, particularly as Orlov (1926a, b) described cells on the lower part of the foregut of Astacus fluviatilis which he presumed to be mechanoreceptors. Such receptors may be involved in the control of the oesophagus movements via the oesophageal and commissural ganglia and would therefore make a profitable subject for study. A further reason for interest in the anterior thoracic area was the likelihood that the well-known series of proprioceptors in the walking legs of decapods (Bush, 1965abc; Cohen, 1965; Alexandrowicz, 1967; Shelton and Laverack, 1968; Clarac, 1968ab) would probably be modified in the structurally and functionally differentiated mouthpart appendages.

This section is concerned with three receptors from the oesophageal-mandibular region of Homarus vulgaris (that is

ventral to the receptors described by Orlov). Following the initial stages of morphological investigation the main aim of this work was to determine the normal stimulus for the receptors and the characteristics of the input from them to the CNS.

2.2.2. Materials and Methods

All the work described in this paper was carried out on specimens of Homarus vulgaris between 20 and 22 cm in length. The animals were obtained locally and kept in tanks of circulating sea water until required.

i. Anatomy.

The methods of Alexandrowicz and Whitear (1957) were used for methylene blue staining. The dissection was started by isolating the thorax and removing the dorsal part of the carapace from it. The stomach was cut away down to the level of the connectives, then the muscles connecting the oesophagus to the lateral wall of the thorax were cut on one side of the animal and the oesophagus deflected to the opposite side. The initial dissection was completed by removing the green gland, bladder, and loose sheets of connective tissue that invest much of the interior of the thorax.

To expose MPR 1 it was necessary to follow the inferior oesophageal and outer labral nerves from their origin at the commissural ganglion and to remove connective tissue from them progressively as

they stained. The receptor cell bodies always lie near the front end of the oesophageal tegumental gland (Yonge, 1924) and have to be carefully isolated from connective tissue which covers them. As soon as the cells were visible the dendrites were exposed by removing the main strand from its position over the receptor strand.

MPR 2 and MPR 3 were more difficult to locate. The dorsal part of the posterior of the oesophagus on the exposed side was isolated from the endophragnal skeleton. Then the skeleton overlying the circumoesophageal commissures was removed back to the sub-oesophageal ganglion. The connective was cut away up to the commissural ganglion. This operation exposed the paragnathal nerve which lies medial to the inner mandibular nerve. The paragnathal nerve was followed to the posterior border of the oesophagus as it stained. At this point it passes below a large blood vessel that runs forward over the tegumental gland. This vessel was carefully removed to expose MPR 2. As the receptor stained further adherent connective tissue was cleared away. The connection of the main receptor strand with the apodeme of the posterior adductor muscle was then cut and the tissues pulled gently apart to expose MPR 3. The best preparations of the third receptor were obtained by splitting the thorax with a medial longitudinal cut at this stage. Following this operation the lower posterior border of the oesophagus was cut so that it could be deflected

further laterally to expose the position where the receptor strand inserts near the paragnath.

ii. Physiology.

Three different preparations were used to obtain recordings from the sense organs. The simplest preparation (type A) was obtained by isolating the thorax and removing the dorsal carapace and stomach (to the level of the connectives) from the more ventral region. The gut contents were washed away with sea water and the green gland and loose connective tissue removed. After another thorough wash in sea water the lower part of the thorax was placed ventral side down in an experimental dish filled with cool (10°C) saline (Pantin, 1962). The outer labral nerve is easily seen on the wall of the oesophagus and recordings of MPR 1 were made from this nerve (either connected normally or disconnected centrally). Destruction of the anterior continuation of the nerve into the labrum was relatively easily accomplished by cutting in the region anterior to the sense organ.

The nerves serving MPR 2 and 3 were exposed by removing the dorsal part of the sternal canal. Two cuts were made longitudinally along the sides of the canal allowing the whole top section to be lifted forward. The muscles connecting this part of the endophragmal skeleton to the oesophagus could then usually be cut without damage to the relevant nerves. Occasionally the wrong nerve trunk was picked up

Initially, the direct supply to the paragnath being especially confusing in the unstained preparation, but test recordings obtained by stimulating the mechanoreceptors in the paragnath show this error clearly.

In this type of preparation if MPR 1 only is investigated it is possible to stimulate the motor nerves to the intact major abductor and lateral adductor muscles. Stimulation and activation of these muscles generally causes a closure of the mandible (that is to say the adductor response dominates).

In type A preparations the large posterior adductor muscles were cut away from their insertions on the dorso-lateral parts of the thorax early in the dissection. Stimulation and contraction of these muscles was therefore impossible. To overcome this problem a second (type B) preparation was used. The anterior thorax was isolated from the remainder of the body and the digestive gland removed from it. The anterior lateral walls of the thorax were removed at a level just above the lower border of the antennae as far back as the anterior border of the insertion of the lateral mandibular adductors (leaving these muscles intact), and then passing dorsally in front of the posterior adductor muscles. If this dissection is carried out properly the muscular elements associated with movements of the mandible are able to perform in their normal manner. The MPR 1 can be uncovered by further dissection of the anterior portion of the oesophageal wall, and MPR 2

and 3 can if necessary be approached by dissection from the posterior border of the thorax by removal of the endopharyngeal skeleton. The geometry of the situation, however, makes electrode placement from the rear or the front rather awkward, and this type of preparation is not recommended for the study of MPR 1 and 2 unless concomitant recording of all three receptors is desired at one time.

The third type of preparation (C) was designed to enable easy recording from MPR 2 and 3, and took advantage of the fact that the nerve trunks supplying these organs lie bilaterally close to the mid-line of the animal. The thorax was isolated and then split into two symmetrical halves with a dorsal cut passing between the bilateral posterior adductors, and a ventral cut that severed the animal in the mid-line between the various appendages. With this procedure one or other hemisection contained workable nerve trunks. It was only rarely that both sides contained usable preparations. The half thorax thus obtained was pinned through the carapace so that the nerve tracts were uppermost and the movements of the mandible unimpeded when the posterior adductor muscle contracted. Stimulation of the other mandibular muscles was not carried out in this type of preparation.

Conventional recording techniques were used. The appropriate nerve was lifted on platinum electrodes into a pool of liquid paraffin

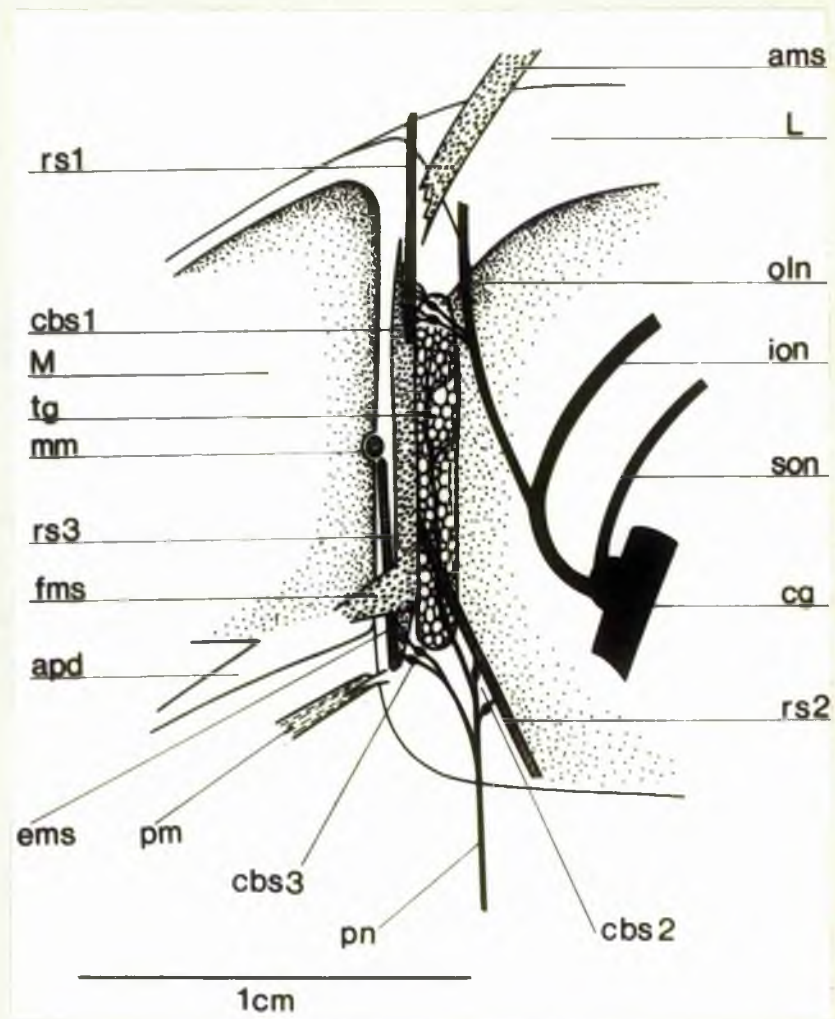
floating on top of the saline that bathed the remainder of the preparation. Amplification was by AC amplifier and display was on a Tektronix 565 oscilloscope (two 3A3 pre-amplifiers). Stimulation of nerve trunks was by rectangular pulses of 0.1 - 1.0 msec duration from Tektronix 160 pulse generators delivered to the motor nerves via an R1 probe, diode system (Coombes, 1965) and platinum wire electrodes. Movement of skeletal elements and muscle tension were recorded by use of an RCA 5734 mechano-electrical transducer. Manual movements were either made by hand with recordings via the transducer or by means of a micro-manipulator fitted with a variable resistance system which accurately monitored movement in the required direction (Shelton and Laverack, 1968).

2.2.3. Anatomy

The structure of the floor of the thorax and the internal surface of the mandible in Homarus is basically similar to that of Astacus as described by Schmidt (1915). The mandible is hinged at two points and is moved by two sets of antagonistic muscles. The three receptors (MPR 1, 2 and 3) are arranged bilaterally in the oesophageal-mandibular region. Figure 2.2.1. shows the positions of the receptors in a preparation. All three receptors are associated with a strand of tissue which spans the antero-posterior length of the oesophagus.

FIGURE 2.2.1.

A diagram of the anterior of the left oesophageal mandibular region of Homarus vulgaris to show the position of MPR 1, 2 and 3 in relation to the main strand. The main strand runs from its anterior insertion (ams) over the labrum (L), passes between the mandible (M) and the tegumental gland (tg) to the rear edge of the mandible where a flap (fms) joins the tissue overlying the apodeme of the posterior adductor muscle of the mandible (apd). A small extension (ems) passes back ventrally from this point to join the thorax near the base of the paragnath. The anchoring positions of the main strand are not shown on the diagram. MPR 1 lies most anteriorly (rs 1 receptor strand 1, cbs 1 MPR 1 receptor cell bodies). This receptor is innervated from the outer lateral nerve (oln) branch of the inferior oesophageal nerve (ion). These nerves, together with the commissural ganglion (cg), and the superior oesophageal nerve (son), lie on the side wall of the deflected oesophagus. (The connective has been removed from both sides of the commissural ganglion). The paragnathal nerve (pn) innervates the other two receptors. MPR 2 lies on the lateral posterior edge of the oesophagus at the level of the base of the sternal canal. It is shown as a single strand joining onto the main strand for simplicity but the actual situation is more complex (rs 2 simplified strand 2, cbs 2 MPR 2 sense cell bodies). MPR 3 lies lateral and ventral to MPR 2. When the mandible is closed its posterior insertion near the base of the paragnath lies below its anterior insertion on the mandible near the insertion of the inner ventral pyloric dilator muscle (imm). The extension of the 1st maxilla which projects as a rod is shown as a marker. The lateral adductor of the coxopodite (pm) inserts in this region and part of the muscle is often attached to this rod. (rs 3 receptor strand 3, cbs 3 MPR 3 sensory cell bodies).




This main strand runs from the ventral insertion of the basal ocular muscles to the mandibular insertion of the apodeme of the posterior adductor muscle of the mandible. At this point a flap leaves the top of the main strand and joins the tissue overlying the apodeme. Anteriorly branches of the main strand anchor it securely to the mandible and the border of the labrum. Along the side of the oesophagus numerous branches run horizontally and ventrally from the main strand into the region of the tegumental gland and the oesophagus. Some branches also run laterally to the mandible. At the point where the flap leaves the top of the main strand to cross over to join the apodeme of the posterior adductor the ventral anchoring strands slope backwards and form a less substantial extension of the main strand. This extension passes back ventrally along the same antero-posterior line as the main strand and joins the skeleton near the thoracic base of the paragnath. The main strand is therefore firmly attached to the mandible and to the oesophageal wall along most of its length. The strand is not elastic because it does not stretch easily but appears to be under constant tension since any cut made in it is followed by immediate retraction of the strand away from the cut. It is probably best compared with the suspensory ligament of the thoracico-coxal receptors (Alexandrowicz, 1967).

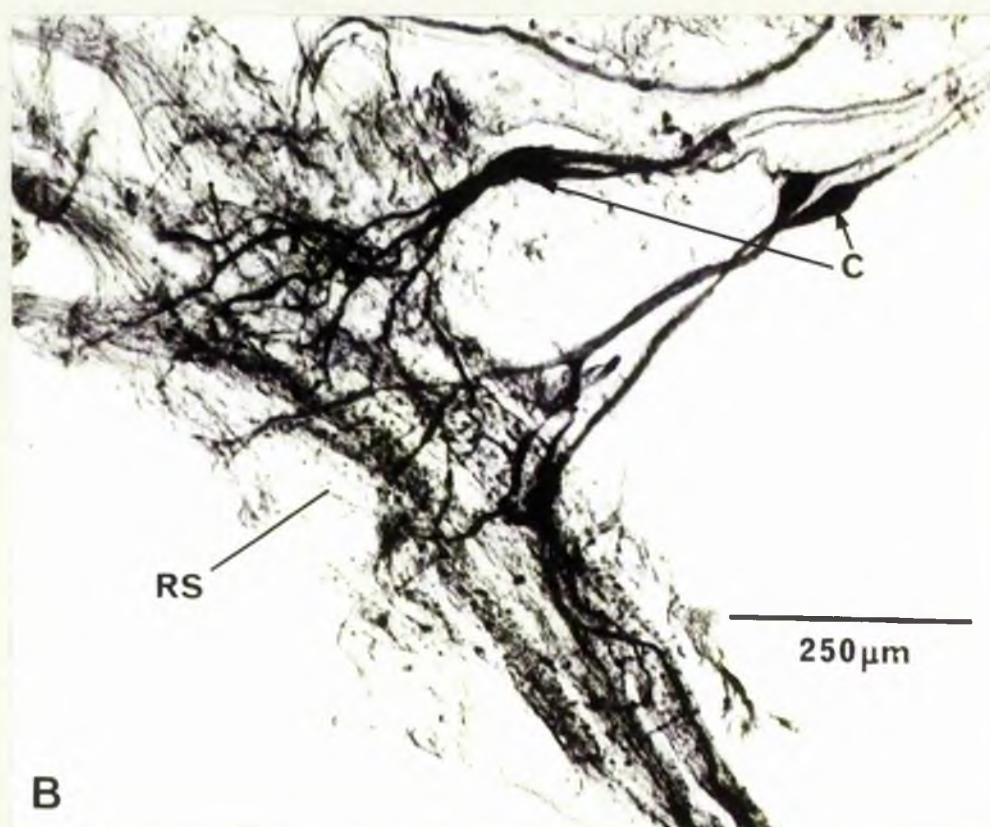
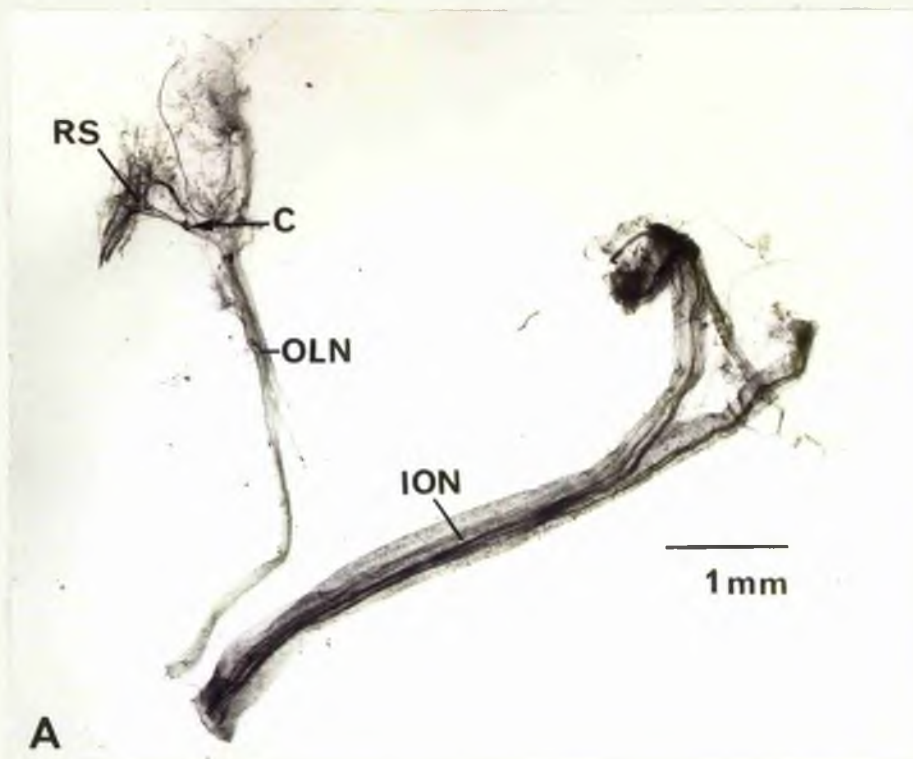
MPR 1 is the most anterior of the receptors. It is innervated from the inferior oesophageal nerve (figure 2.2.1.) The inferior oesophageal nerves originate at the commissural ganglion on each side of the oesophagus and run to the medial oesophageal ganglion (Orlov, 1926a, b). Soon after leaving the commissural ganglion each inferior oesophageal nerve gives rise to a small nerve trunk that courses forward to innervate the labrum (anterior border of the mouth). A much larger nerve trunk which also runs anteriorly arises close to the mid-line of the animal from the inferior oesophageal nerve. The small outer labral nerves in turn each have a further branch that progresses laterally and runs toward the anterior corner of the mandible. This branch contains a small group (4-5) of nerve cells which innervate the posterior part of a discrete strand of tissue (figure 2.2.2A). This strand runs straight forward from the ventral side of the main strand to the carapace near the anterior hinge of the mandible. It has a smaller diameter than the main strand. This receptor strand does not appear to be muscular (having no striations and no motor innervation) and is probably analogous to the elastic strand receptors of the thoracico-coxal joints of the walking legs. The receptor strand stretches easily and returns to its original length after large extensions.

The sensory cell bodies lie close to the strand and the axons proceed undivided to the circumoesophageal connective at the commie-

FIGURE 2.2.2.



Photomicrographs of a methylene blue stained preparation of MPR 1.
A: A low power picture to show the relation of the receptor strand (RS) and cells (C) to the outer labral (OLN) and inferior oesophageal nerves (ION). B: A more detailed view of the cells (C) and their endings on the receptor strand (RS).



sural ganglion. Peripherally the dendritic region is complex (figure 2.2.2B). Each cell gives rise to a multiplicate dendrite tree which ramifies on the receptor strand. Some branches may also innervate the connective tissue surrounding the receptor strand, a situation paralleled in the cockroach hypopharyngeal receptors (Moulins, 1966). The precise organisation of the dendritic branches is variable; some cells are classically bipolar in shape with only distant branching of the dendrites; others are multipolar with one branch as axon and a variable number of other processes innervating the receptor strand.

MPR 2 and MPR 3 are innervated by a branch of the paragnathal nerve. This nerve courses forward near the medial line from the sub-oesophageal ganglion. One portion runs to the posterior part of the oesophagus; this branch divides and commonly two further branches pass ventrally to innervate MPR 3 and one branch goes forward to innervate MPR 2 and the area over the tegumental gland.

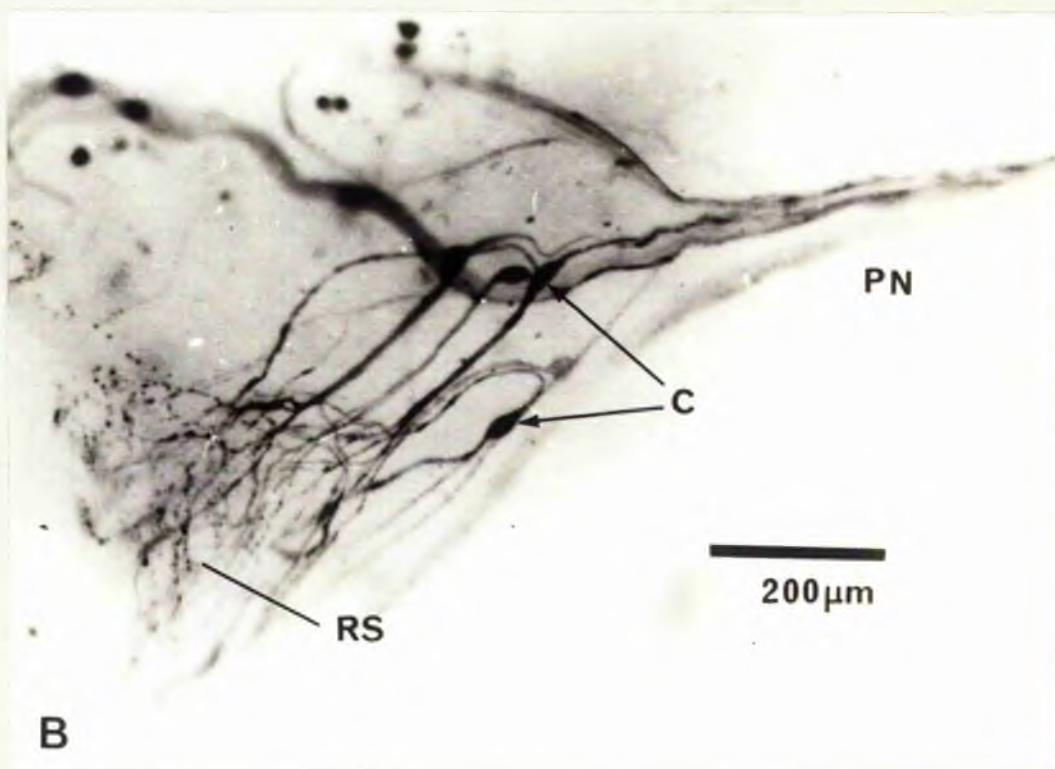
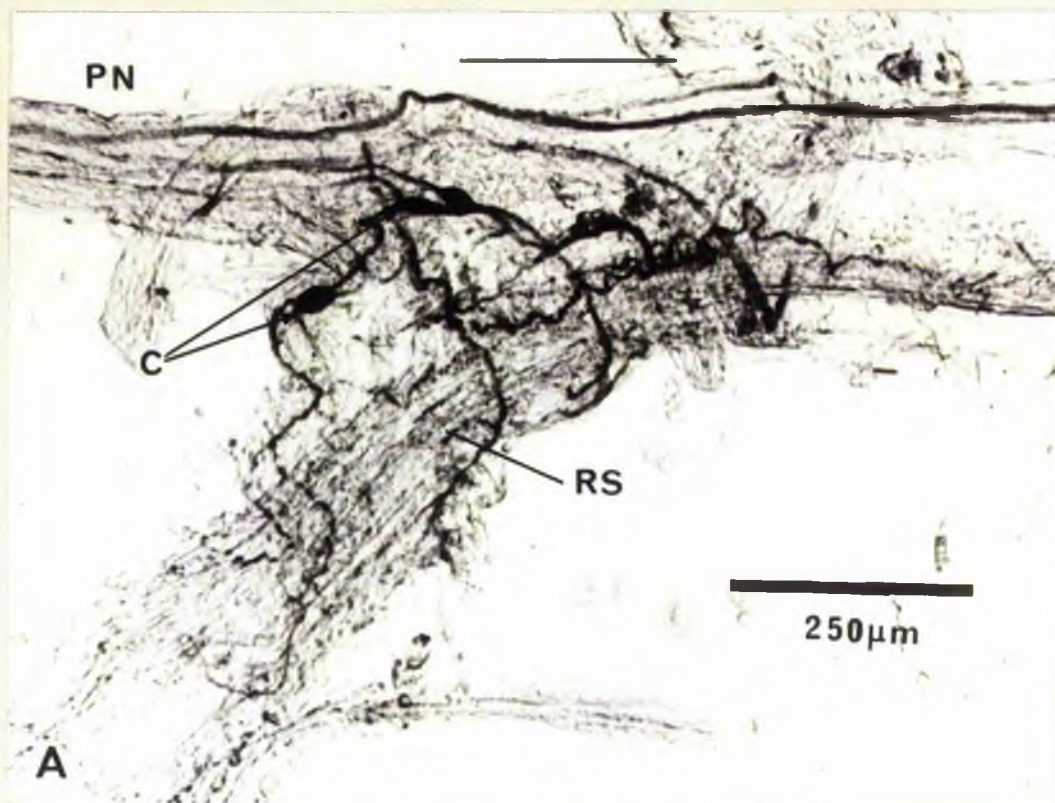
MPR 2 is not so obvious or as discrete a structure as MPR 1 or 3. Between the oesophagus and the main strand there are many fibres of connective tissue. Some of these fibres run forward from the posterior border of the oesophagus, at the level of the ventral part of the sternal canal, towards the main strand at a position just behind the insertion of the inner ventral pyloric dilator muscle on the mandible. These strands

are innervated by the peripheral processes of cells in the paragnathal nerve which lie on or near the strands (figure 2.2.3A). A feature of these peripheral processes is that they often run some way in the strands with little branching. In general there is a small amount of branching of these dendrites compared with MPR 1. The cells are not usually placed at the ends of the strands and processes may run both backwards and forwards from the cells. There are usually about 5-10 cells to be seen. From the position of the cells the paragnathal nerve runs forward and ramifies in the tissues overlying the tegumental gland.

MPR 3 is situated on a more discrete elastic strand rather like MPR 1. Its posterior insertion is near the thoracic base of the paragnath close to the insertion of the posterior extension of the main strand. From this insertion it runs forward lateral to the main strand extension but is apparently connected with it in places. The receptor strand passes below the flap of the main strand and inserts on the mandible near the insertion of the inner ventral pyloric dilator muscle. This muscle runs from its mandibular insertion to the ventral side of the pyloric stomach. The external ventral pyloric dilator muscles are shorter, they insert on the same part of the stomach but run from the junction of the dorsal part of the sternal canal with the posterior part of the oesophagus. The branches of the paragnathal nerve insert on

FIGURE 2.2.3.

Photomicrographs of methylene blue preparations of MPR 2 and 3.
A: MPR 2 neurones (C) in the paragnathal nerve (PN) innervating
a receptor strand (RS). The paragnathal nerve then runs forward
(arrow). **B:** MPR 3 neurones (C) from the paragnathal nerve (PN),
are shown innervating the receptor strand (RS). In situ.



the posterior end of the strand but nerve fibres run anteriorly along the strand. There appear to be more cells than in MPR 1. The complex peripheral ramifications of the dendrites shown in figure 2.2.3B are typical of most of these receptor neurones.

In some preparations we have seen other bipolar cells both in the outer labral nerves between MPR 1 and the labrum and in the paragnathal nerve over the tegumental gland. These cells do not appear to be consistent in position.

2.2.4. Physiology

Electrical activity is recordable in all the mouth part receptor nerves after dissection. It differs in intensity and form in MPR 1 and the rear pair.

i. MPR 1. Care must be taken not to stretch the nerve as the extreme sensitivity of the organ will lead to spurious results. The organ functions when completely isolated from the remainder of the nervous system; that is, with the circumoesophageal commissures cut posteriorly and anteriorly, with the labral nerve severed to remove any influence of structures in the labrum, and with the outer labral nerve isolated from the commissures. No efferent activity from the CNS is noticeable.

MPR 1 is most notable for the regular activity of some of the com-

power units. In each of the figures shown of recordings from this organ it will be seen that at least one, often two, and sometimes three units fire with great regularity. The frequency of discharge is maintained over long periods of time (several minutes) without variation, and may continue for up to three hours (when the longest experiment was terminated).

Superimposed upon these regular discharges are phasic bursts that occur upon movement of the main strand. Figure 2.2.4 shows the normal discharge in this nerve (figure 2.2.4A), and the response to small imposed movements of the mandible (figure 2.2.4B). In the latter case the mandible was closed several times, and phasic units discharged a volley with each closure. Adaptation was rapid, and phasic units ceased firing immediately the movement was completed.

These phasic responses to passive movement of the main strand are mimicked by more natural events as shown in figure 2.2.5.

Stimulation of the nerve supplying the posterior mandibular adductor muscle leads to raising of the mandible (normal raising and closure of the appendage). Normal background activity (figure 2.2.5A) is interrupted by rapid closure of the mandible, and phasic receptors are stimulated. These adapt before the muscle relaxes (figure 2.2.5B). Essentially similar results are obtained in experiments involving the stimulation of the major adductor and lateral adductor muscles

FIGURE 2.2.4.

Recordings from MPR 1, type A preparation. The response to closure of the mandible caused by manual movement of the apodeme of the posterior adductor muscle. Top line of each record indicates movement of mandible as indicated by the RCA transducer placed on the skeletal division between the major abductor and lateral adductor muscles. Downward movement of line shows closure of mandible. Bottom line of each trace shows activity of MPR 1.

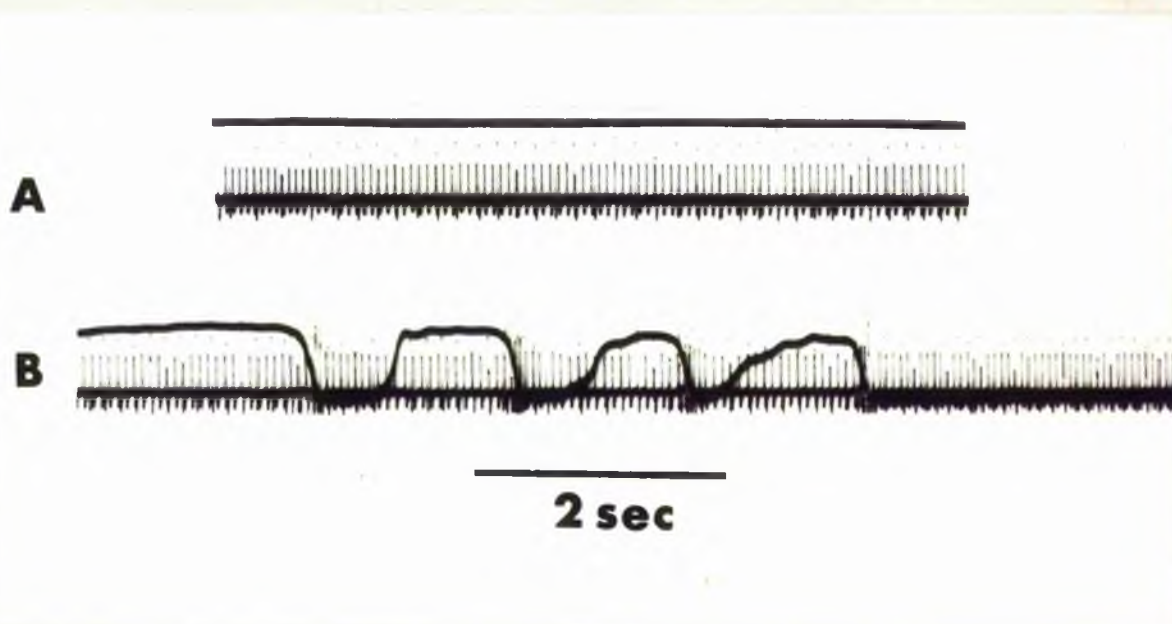
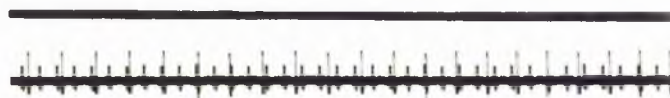


FIGURE 2.2.5.

Recordings from MPR 1, type B preparation. The response to closure of the mandible brought about by electrical stimulation of the nerves supplying mandibular muscles. Top line shows mandibular movement recorded as in figure 2.2.4., except that closure is shown by an upward movement of the line. Bottom line of record shows stimulus (B and C) and the middle line is recording of organ responses. A. No movement. B. Movement brought about by contraction of posterior adductor muscles. C. Movement caused by lateral adductor (and apposition of major abductor) muscles. In this example the stimulation caused a small slow closure.

A



B



C



1sec

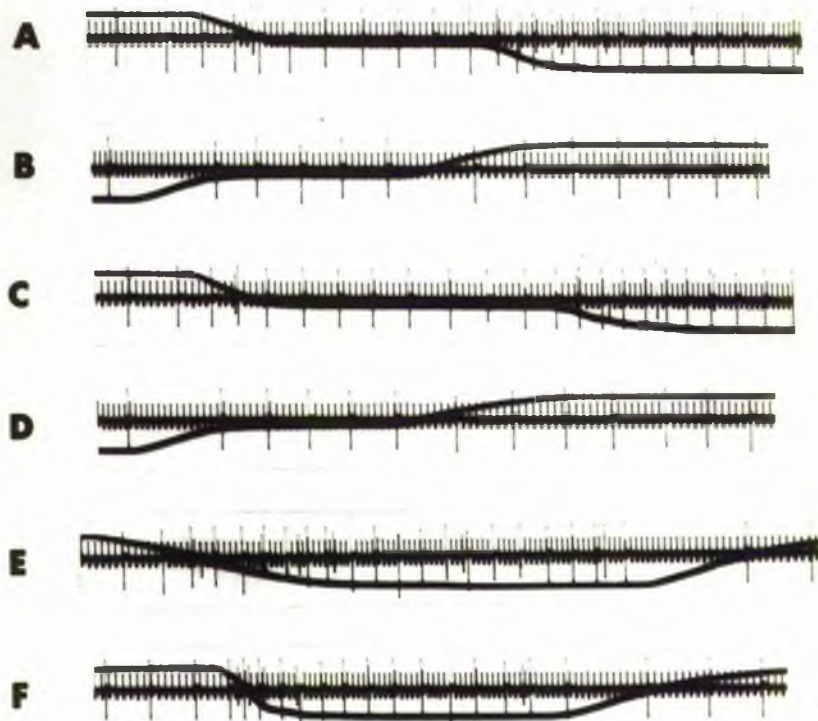
(figure 2.2.5C). These slow movements are not monitored by phasic units.

Events in the tonic units can be followed in figure 2.2.6. Closure of the mandible is shown by movement of the trace down across the record. In this experiment two units were firing regularly and at slightly different frequencies, but in certain places on the record the two fire at the same moment of time, and the spikes summate to give the appearance of a third larger spike. In figure 2.2.6A and figure 2.2.6C the mandible is closed in two steps, and at both steps the frequency of the tonic units changes, becoming slightly faster with increased positional change (see also figure 2.2.7A). The most dramatic demonstration of this cannot be shown in here, that is the modulation of the audio-monitor which is run concurrently with all experiments. The frequency change is most striking when audible.

The reverse procedure, allowing the mandible to re-open is monitored by a slight decrease in frequency at each step (figure 2.2.6B and 2.2.6D). At the beginning of each closure movement the phasic units may fire briefly, but they do not respond to opening (figure 2.2.6F). The large tonic unit is clearly sensitive to rate of movement (figures 2.2.6E, F; 2.2.7B). This effect is not as marked in the small unit. Further experiments on this aspect are probably possible using only the isolated receptor system. All

FIGURE 2.2.6.

Recordings from MPR 1, type A preparation. The response to closure of the mandible caused by manual movements of the apodeme of the posterior adductor muscle. Downward movements of line indicate closure of the mandible recorded directly via the variable resistance system on the micromanipulator. A-D. Continuous recordings of steplike movements (see also figure 2.2.7A). E and F. A slow and fast movement through a large part of the total range of possible mandibular movement (see also figure 2.2.7B).



1sec

9

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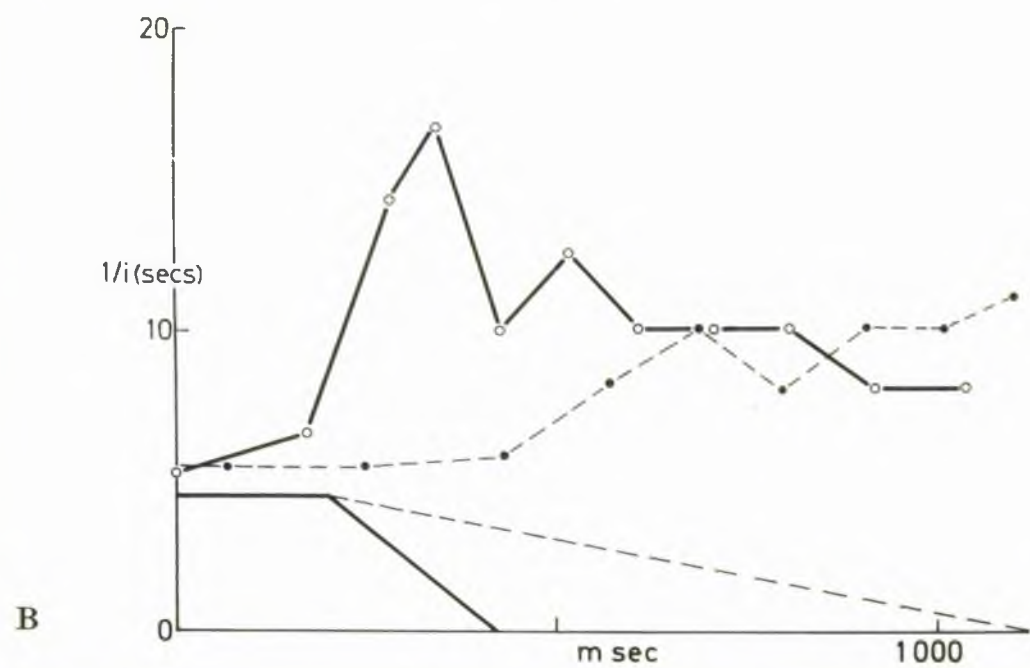
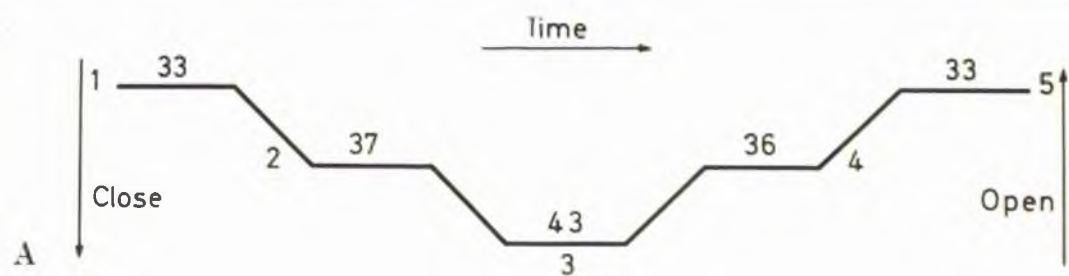
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FIGURE 2.2.7.

Graphs of responses of MPR 1 shown in figure 2.2.6. A. Representation of spike frequency per second for the small tonic unit for three step positions (closing 1,2,3; opening 3,4,5). These averages were taken from a number of complete series such as A and B in figure 2.2.6. B. Reciprocal of time between successive spikes plotted at the mid-point between successive spikes for the response of the large unit to a slow (figure 2.2.6E) and fast (figure 2.2.6F) closure of the mandible. Slow closure and response are shown as broken lines; rapid closure as full line.



the receptors studied were also sensitive to small spontaneous oesophageal movements which were well within the range normally observed in dissected preparations.

II. MP2 2 and 3. These organs may be stimulated by manually stretching the main strand, particularly at the anterior end of the thorax. Similar responses occur if the apodeme of the posterior mandibular adductor is moved along its axis. Movements of the mandible, either closing or opening, are monitored by responses of MP2 2 and 3. These responses may be phasic or phasic-tonic. Figure 2.2.8 (A, B) shows activity in two units, one responding to closing of the mandible, the other firing when the mandible is in the relaxed position (open). These responses alternate when the movement is cyclic (figure 2.2.8A). Figure 2.2.8B is a faster record of the same event. It should be noted that the larger unit in this case (discharging upon closure) is movement and velocity sensitive, adapting when held in a maintained position. The second smaller unit (firing in the maintained open position), is position sensitive, and only fires when movement is maximal. Figure 2.2.8C and 2.2.8D show a different unit that fires when the mandible closes, but which is position sensitive, and very slightly velocity sensitive.

Stimulation in the natural situation is shown in figure 2.2.9. In this case the motor nerve supplying the posterior adductor muscle

FIGURE 2.2.8.

Recordings of MPR 2 and 3, type A preparation. Responses to closure of the mandible caused by movements of the skeletal division between the major abductor and lateral adductors. Bottom line in A and B, top line in C and D are direct recordings of micro-manipulator movements (via variable resistance system). Downward movement indicates closure. A. Two units, one responding to closure the other to opening. B. This response filmed at five times the speed of A. Both traces in C and D show another unit from a different preparation. Time scale = 2 sec for A, C, D and 400 msec for B.

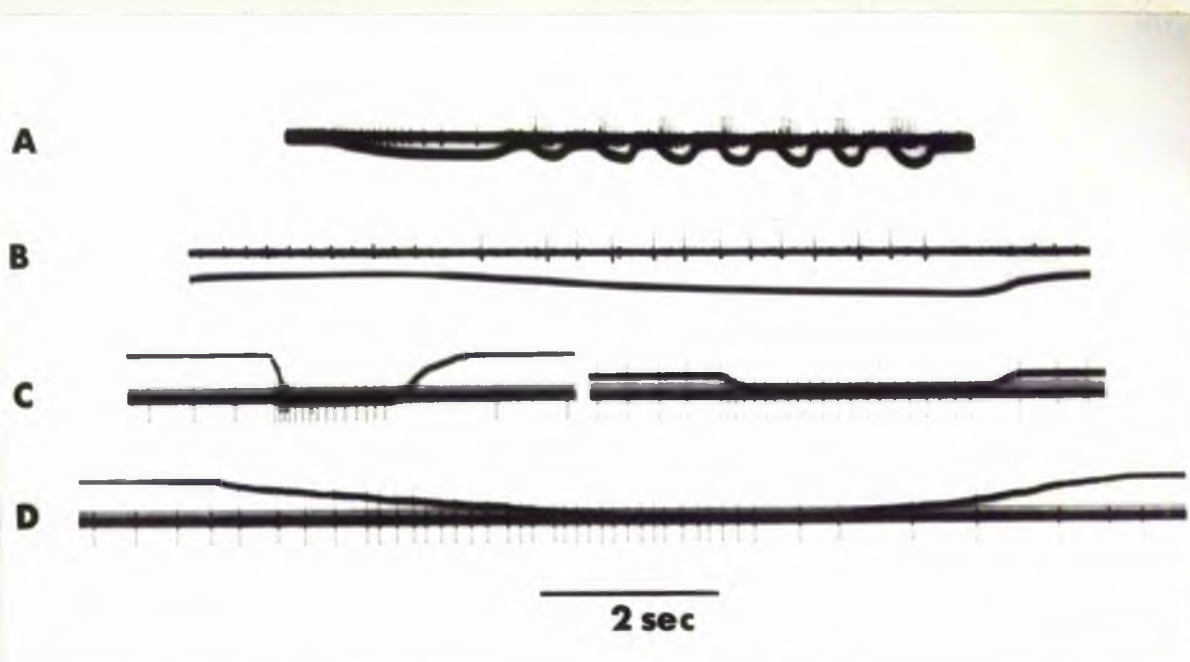


FIGURE 2.2.9.

Recordings from MPR 2 and 3, type C preparation. Responses to movements of the mandible caused by electrical stimulation of the posterior adductor muscle. The top line in all traces shows the recordings from the receptors. The middle line shows the stimulus to the motor axons and the bottom line shows the movement of the muscle recorded via the RCA transducer (closure is shown by a downward movement in A, B and C and an upward movement in E). The time mark refers to C, D and E. A and B were filmed at one fifth of this speed.

A



B



C



D



E



1sec

was electrically stimulated, contraction of this muscle lifting the mandible. Figure 2.2.9A shows different periods of duration of stimulation, and hence duration and magnitude of mandibular closure (lifting). Phasic units predominate, but a single tonic unit is also present. A tonic unit is also seen in figure 2.2.9B, but the frequency in this case is modulated by mandible movement. It will be seen that phasic receptors again fire on closure, and at least one of these is rather slow adapting since it continues to fire during the period of maintained position. The prevailing tonic discharge, however, is interrupted by movement at all stages, and remains silent until the mandible returns to the original position, when it continues in the same frequency as previously. This is shown more clearly in figure 2.2.9C. A further type of response is shown as a single unit record in figure 2.2.9D and 2.2.9E. A tonically discharging unit shows an increase in frequency when the mandible closes.

Although the axons of these organs run closely together and hence are almost inseparable in situ, it is possible to selectively destroy the organs one at a time. If either organ is destroyed there is little difference in the recordable response to mechanical stimulation. This presumably indicates a plurality of response in which both sense organs respond to similar stimuli. In view of the involvements of the

main strand with both receptors this is not surprising. There does, however, seem to be a certain polarity of response characteristics in that MPR 3 responds to mandibular movements more dramatically than does MPR 2. MPR 2 on the other hand, responds to movements of the oesophagus more readily. There may thus be slight differences in input from these two organs, but in general it can be stated that any distortion of the main strand particularly in a fore and aft direction can be correlated with activity in these sense organs. MPR 3 responds to movements of the paragnath as well, but these movements were not evoked by experimental stimulation of muscles and it is not known if paragnath responses occur naturally.

Part 3. Discussion and Conclusions

Section 3.1. Sensory Systems

3.1.1. The Posterior Stomach Nerve

The mode of action of the gastric mill in the decapod crustacea has been the subject of a long debate (e.g. Mocquard, 1883; Pearson, 1908; Patwardhan, 1935; Reddy, 1935). The argument has centred on the relative importance of the anterior and posterior gastric muscles in the movements of the mill. At the present time it is only possible to draw tentative conclusions on this subject because of the difficulty of making open Cancer preparations function over any length of time. Previous descriptions of the movements have mostly followed Mocquard's (1883) observations on some crabs with transparent cuticles. He states that the major feature is a forward motion of the mesocardiac ossicle from the resting position caused by contraction of the anterior gastric muscles. This may be the major movement in Homarus but it was not often seen in Cancer. Mocquard himself was careful to state that his observations may not have covered all the possible movements of the mill. In the experiments reported here it was difficult to judge the effect of the swelling of the cardiac stomach which usually occurs when the carapace is removed, on the activity of the gastric muscles. The

important point is that the cycle of movements which is described does occur in most active preparations and thus constitutes a part of the normal activity of the mill.

Repetitive stimulation of the nerves which originate from the stomatogastric ganglion and run posteriorly over the dorsal surface of the mill evokes movements which are very similar to the normal cycle of movements. The response in the p.s.n. indicates that the mechanoreceptors in the nerve function during normal movements of the gastric mill. The lack of response to movements of the pyloric region of the stomach shows that the p.s.n. does not directly monitor the passage of food from the cardiac to the pyloric stomach.

The small amount of residual activity in the section of the p.s.n. central to a cut which leaves the main group of cells on the distal side, indicates that the p.s.n. contains few motor fibres unless the neurones have failed centrally. The failure of stimulation of the section of the p.s.n. distal to a cut, to elicit movements of the gut also shows that the p.s.n. has no motor neurones unless the neuromuscular junctions have failed. These observations were made on the dorsal surface of the gastric mill and it is possible, but unlikely, that small movements of other parts of the mill were not seen. I conclude that the p.s.n. carries no motor fibres innervating the gut.

The possibility that the nerve carries inhibitory fibres has also been eliminated because concurrent stimulations of the p.s.n. and the motor nerves from the stomatogastric ganglion had no effect on the movements. It is not likely that the p.s.n. contains chemoreceptors or innervates glandular tissue because the fibres do not end in the gut cuticle and there are no glands to be seen in this region.

I have shown that a p.s.n. exists in species of all the sections of the decapods which have a complex gastric mill, that is in the sub-class Reptantia, sections Anomura, Brachyura and Macrura. It would be worthwhile to make a thorough investigation of the species of the sub-class Natantia which do not have a gastric mill (section Caridea), or which have a simple mill (section Penaeidae). This might help to establish the function of the p.s.n. in the species examined here. A preliminary examination of a small number of Penaeids (Metapenaeus) and Stomatopods (Squilla) has so far failed to reveal any similar nerve. This suggests that the p.s.n. is directly involved in the function of the mill. The classification and summary of the structure of the gastric mill in the decapods is taken from Barnes (1963).

Heath's failure to describe cell bodies in the p.s.n. of Pugettia

is not surprising as he used toluidine blue to stain nerve sheaths. It is more difficult to understand the lack of a description of a p.s.n. in the work of Keim (1915) on Astacus, Orlov (1926) on Astacus, and Chaudonneret (1956) on Cambarus. Perhaps the arrangement in Astacus is somewhat different to that in Homarus and Palinurus though this seems unlikely. The innervation of the gastric mill via the mandibular nerves might seem curious. The theory proposed by Patwardhan (1935) is pertinent in this context. This author suggested that there was a correlation between the decline in the external mandibular mastication apparatus, the increased development of the gastric mill and the way of life of the various groups of decapods. He noted that in the animals without complex gastric mills (Natantia) the food is masticated mainly or partly in the buccal cavity by the well-developed mandibles, whereas in the Reptantia the food is masticated in the complex gastric mill and the mandibles are much simpler. He suggested that this change was due to the Reptantia needing to obtain food quickly and then retiring to shelter to eat it. It is therefore (on his theory) less surprising that the gastric mill is innervated via the mandibular nerves, as it can be considered as one of the series of mouth parts.

The anatomy of the p.s.n. suggests that the cells in it are sensory and this is confirmed by the intracellular recordings from the cells in

the main group in Homarus. The cell body and fibre counts indicate that the peripheral processes from the cells in the main group make up at least a large percentage of the fibres in the p.s.n. which run to the gastric mill. This, combined with the similarity of the electrical activity which can be recorded central and distal to the main group of cells, indicates that many processes which are morphologically dendrites carry propagated action potentials as has been found in other decapod mechanoreceptors (Mellon & Kennedy, 1964; Mendelson, 1966; Hartman & Boettiger, 1967).

The sensory neurones in the p.s.n. are probably type IB receptors (see appendix) having branched dendrites which end with no obvious terminal specialization, but it is not possible to be sure that some of the dendrites do not end on muscles. I have attempted to cut the l.v.n. and m.v.n. in Homarus in the hope that they would degenerate and allow an investigation of the p.s.n. endings without the complications of the anastomoses. However as might be expected from the report of Hoy, Bittner and Kennedy (1967) the neurones in the l.v.n. and m.v.n. were in good condition two weeks after the operation. The units observed in the p.s.n. have all been varieties of the intermediate phaso-tonic type such as the unusual sensory neurone in the stomatogastric ganglion of the crayfish reported by Larimer & Kennedy (1966), and the telson

receptor of Barth (1964). Distortions of the connective tissue caused by movements of the ossicles must result in the generation of action potentials very close to the gut. The fact that the fibres branch widely on the gut might be expected to impose difficulties for the CNS in the interpretation of the sensory information. This topic was investigated by Pabst & Kennedy (1967) for the crayfish cutaneous mechanoreceptors which have many similar features.

The fact that repetitive stimulation of the p.s.n. alters the output in the nerves from the stomatogastric ganglion should allow a more precise determination of the function of the information in the CNS. Experiments to determine the function of the p.s.n. sensory system are probably best carried out on Homarus because Maynard (1966) has already analysed some of the output from the stomatogastric ganglion, and the part of the stomatogastric nervous system between the commissural and oesophageal ganglia is well known and approachable. There is also a good deal of pertinent information on neuronal pathways in Homarus (Allen, 1894) and Astacus (Orlov, 1926-29).

It may also be possible to record from the stomatogastric nerve before it enters the stomatogastric ganglion in female Cancer without too much destruction of the anterior part of the stomatogastric nervous system. Experiments using this recording site and stimulation of the

p.s.n. may provide information on the interneurons involved in any activity. The fact that in a few preparations stimulation of the central end of the p.s.n. caused normal gut movements indicates that this is an important input for the system.

A point of comparison with Maynard's work on Homarus concerns the inhibition of the m unit which he found was associated with increased l and a unit activity as the stimulus to the stomatogastric nerve was increased (section 1.2). I found that although the effects of p.s.n. stimulation on the units in the d.v.n. was variable often the l unit activity increased. Stimulation of the p.s.n. in Cancer is definitely associated with inhibition of the m unit. This confirms that the p.s.n. sensory system is a normal input to the stomatogastric ganglia.

Clearly the biggest problem encountered in working with this system was the difficulty of making the mill function in the animals which had their carapaces removed. Perfusion of the anterior aorta has been tried in a number of experiments with no marked success. Attempts to cut the possibly inhibitory nerves from the brain (see section 3.2) have also been made but dissection in this region of Cancer is not simple and again no success was achieved. En passant stimulation of the intact stomatogastric nerve has some effect but it does not result in continued activity of the mill. Perhaps the only solution

is to keep an operated crab in the experimental dish over a long period. If only the chelae of the animal are removed (by autotomy or surgically plus cauterisation) the animals live in sea water for some time. By efficient sterilisation procedures, the fully dissected animal could be expected to live for a long time. By starvation of the animal before the operation and feeding after a few days of acclimatisation continued activity of the mill might be obtained. This would allow the necessary simple experiments to elucidate the function of the p.s.n. to be made. Such experiments as cutting both p.s.ns. during movements, recording from receptors during natural movement, recording output from the ganglion, adding out of phase stimulation to the p.s.n. and so on. Another possible approach is to insert electrodes through the carapace and record activity in the 'normal' animal before and after various operations on the stomatogastric nervous system.

3.1.2. The Oesophageal - Mandibular Receptors

As anticipated the mandibular receptors described here differ in their anatomical properties from the thoracico-coxal receptors which lie in a comparable position in the walking legs. The most noticeable difference is in the lack of a chordotonal organ. This contrasts with the situation described for the thoracico-coxal receptors of the walking legs (Alexandrowicz, 1967) but studies on the thoracico-coxal region of

the 3rd maxilliped reveal that the chordotonal organ is also missing in this limb (Clarac, Wales, Laverack & Dando, 1969a).

The receptors of the mandibular region are all associated with a major structural ligament that stretches from the epistome almost to the ventral anterior border of the endophragmal skeleton. The receptor neurones rarely send dendrites onto this strand which is therefore mainly an accessory structure. The sensory cells are instead inserted on much more elastic strands that bear precise anatomical relationships to the main strand. The main strand is normally held in a state of tension across the floor of the thorax. It is taut and retracts immediately away from any place where a cut is made. It is possible to stretch the strand mechanically to a small extent. In the intact preparation such stretching, and subsequent relaxation, is accomplished by movement of the mandible in the opening and closing movements that occur during feeding, and by movements of the very mobile oesophagus. As the receptor strands are deformed differentially by movement of the main strand it is assumed that such movements of the main strand are partly responsible for the action of the sense organs. Clearly as each receptor strand also has one end attached to a mobile structure movements of these attachments away from the main strand will cause differential responses of the sense organs. For example MPR 1 can be activated by normal movements of the oesophagus (pulling on the

central part of the main strand) and by mandibular movements (pulling on the main strand and the anterior portion of the receptor strand).

MPR 2 can also be activated by oesophageal movements, probably predominantly by such movements. The input from these receptors alone therefore is not precise and probably only signals that events are taking place around the mouth.

Morphologically the MPR neurones are of a type 2B (see appendix) and although the dendrites are relatively short MPR 1 and 3 resemble the N cells of the dorsal thorax (Alexandrowicz, 1952). Physiologically the MPR cells are dissimilar to the thoracico-coxal muscle receptor neurones of Carcinus (Bush, Ripley, Roberts, 1968; Bush and Roberts, 1968) which usually respond to mechanical stimulation by graded potentials that are conducted by the dendrites to the CNS. In the mandibular receptors the axons certainly conduct action potentials to the CNS.

The observation that both phasic and tonic units occur in MPR 1 cannot be correlated at present with any distinctive morphological features. It is possible that there are different anatomical insertions of the dendrites on the receptor strand as reported for the PD organ of the walking legs (Hartman and Boettiger, 1967) but this seems unlikely. From Whitear's work on the leg thoracico-coxal receptors (1965) it is not expected that different unit responses will be paralleled

by modifications of fine structure.

The types of unit observed in the MPR series are similar to those previously described for other situations, as in the leg chordotonal organs or statocysts. In MPR 1, the best studied example, there are almost pure position fibres, fibres that respond more to movement but have also a positional response, and purely phasic movement units that are rate sensitive. This information is gained from a small (4-5) number of neurones. MPR 2 and 3 seem to contain more units that are phasic and velocity sensitive than other types.

If these internal mechanoreceptors do have a proprioceptive function it seems probable that it would be to affect the mandible, oesophagus and labrum via the commissural and sub-oesophageal ganglia, or the stomach via the stomatogastric ganglion. A further stage in the specific investigation of these receptors would be to determine which if any of these effects were evident and then to investigate them in detail.

3.1.3. Conclusions on Sensory Receptors Innervating the Foregut

Two of the major internal mechanoreceptor organs on the foregut of the decapods have been described here. Both contain multiterminal neurones similar to the previously described 'k' cells. It is known from the work of Orlov and Allen and from my observations that there

are scattered (probable) mechanoreceptor neurones of a similar type on the oesophagus and to a lesser extent on the cardiac stomach. Physiological studies on these neurones will probably prove to be difficult. The labrum is innervated by numerous large neurones which usually have one long dendrite with a multiterminal ending in the hypodermis (c.f. Pabst & Kennedy, 1967). Electrical recordings show that these labral neurones are unusual in that they have no resting discharge but fire phasically (often at high rates) when the labrum is moved. Tonic information is supplied by MPR 1 neurones which increase their firing rate when the labrum moves into the mouth and decrease their rate when the labrum moves away from the mouth (Moulins, Dando and Laverack, 1969). This of course adds yet more complexity to the information supplied by MPR 1 to the CNS.

On the pyloric region of the stomach of Homarus and Nephrops there are often a small number of multiterminal cells to be seen in the nerves just posterior to the p.s.n. anastomoses with the l.v.n. (figure 2.1.7.). It is not certain that these cells are equivalent to Orlov's pyloric sensory cells (figure 1.2.1.) in Astacus. It is also not clear if these cells have axons down the side wall of the gut to the commissural ganglion or back in the l.v.n. to the stomatogastric ganglion. Physiological investigations of these neurones would appear to

be possible. If any of the hairs of the interior of the gut are innervated by mechanoreceptor neurones it may also be possible to record from these cells. However if the neurones are chemoreceptors the difficulty of recording is much greater. Because of the close association of the mandible and lower oesophagus, it is also necessary here to note the overlap of part of the heavy innervation of this appendage. What is now known of the foregut sensory systems is summarised in table 3.1.1.

Clearly the next step in the investigation is to determine the operative stimulus and input characteristics of the pyloric stomach mechanoreceptors. This would also apply to any groups of innervated hairs which could be studied. With this information on the major internal mechanoreceptors it should be possible to turn then to the functions of the commissural and oesophageal ganglia and thus to the input to the stomatogastric ganglion.

Section 3.2. Further Work

3.2.1. Ganglia Functions and Actions of Sensory Systems

The first point that should be made here is that it is somewhat artificial to remove the stomatogastric nervous system from the rest of the nervous system and study it in isolation. The commissural ganglia sit on the connectives and no doubt are under the influence of

Table 3.1.1.

Summary of Receptors Innervating the Foregut

<u>Neurones</u>	<u>Nerve</u>	<u>Axon to</u>	<u>Chemo- receptor</u>	<u>Mechanoreceptor</u>
Large multiterminal cells in labrum. Probably not numerous. Type 1B	outer labral	commissural (?)		phasic responses to labral movements.
Large multiterminal cells in labrum. Numerous. Type 1B	inner labral	commissural (?)		phasic responses to labral movements.
Small uniterminal cells. Probably numerous.	inner labral	commissural (?) probable		
10-15 multiterminal cells on posterior mandibular hinge. Type 1B	posterior stomach	commissural (?) sub-oesophageal (?)		range of phaso-tonic responses to mandibu- lar movements.
4-5 large multi- terminal cells. Type 2B. MPR 1	outer labral	commissural (?)		mainly tonic responses to movements of structures around mouth.
10-15 large multi- terminal cells. Type 2B. MPR 2	paragnathal	sub-oesophageal (?)		range of responses to movements of struc- tures around mouth.

10-20 large multi-terminal cells. Type 2B. MPR 3	paragnathal sub-oesophageal (?)	range of responses to movements of structures around mouth.
Scattered large multiterminal cells on oesophagus. Not numerous. Type 1B	superior commissural oesophageal	probably respond to oesophageal movements.
Scattered groups of 3-5 small unit-terminal neurones.	superior commissural oesophageal	probable
Bilateral group of 100+ small unit-terminal neurones.	superior commissural oesophageal	probable
Scattered large multiterminal cells on cardiac stomach. Not numerous. Type 1B.	superior commissural oesophageal	probably respond to stomach movements.
2-3 large multi-terminal cells in stomatogastric ganglion. Type 1B	stomato-gastric both commissurals	phaso-tonic responses to movement of cardiac stomach.
80+ large multi-terminal cells	posterior stomach commissural (?) stomatogastric (?)	range of phaso-tonic responses to movements of gastric mill.

Small number of	postero-	commissural (?)	movements of pyloric
large multiterminal	lateral (?)	stomatogastric (?)	stomach or hindgut (?)
cells on pyloric	lateral ven-		
stomach	tricular (?)		

Innervated hairs

possible possible

Notes

1. The information is almost completely for *Macrura* alone.
2. In this table a large neurone means a cell body which in one dimension is about 40-60µm. As most of the cells are bipolars this is not a difficult standard to apply to stained preparations. Similarly a small cell is about 10-15µm in length.
3. A group which is called numerous contains at least 50 neurones.
4. A question mark indicates that the information given is probable but not certain.

interneurones from the supra-oesophageal ganglion. (It is certainly possible to produce activity in the oesophagus by stimulation of the anterior part of the connectives). The oesophageal ganglion has a direct connection with the supra-oesophageal ganglion by the ventral median nerve. Also the mouthparts are innervated from the sub-oesophageal ganglion and it is therefore to be expected that this ganglion also regulates the activities of the commissural ganglia. In particular the operation of the mouth region must be closely linked with that of the mandibles.

Nevertheless I have confirmed that a commissural ganglion can maintain a discharge indistinguishable from the normal activity for a long time when completely isolated from the central nervous system. It is also possible to show that this output is correlated with movements of the oesophagus and labrum. Each commissural ganglion also sends a constant bombardment to the oesophageal ganglion. This diffuse ganglion is more difficult to understand. The simplest initial experiments to carry out are to isolate the ganglion and to study any spontaneous activity which is presumably due to cells in the ganglion. I have found that rhythmically firing units send information to the supra-oesophageal ganglion via the ventral median nerve and up the stomatogastric nerve (possibly to the stomatogastric ganglion). Stimulation

of the superior and inferior oesophageal nerves (from the commissural ganglia) evoke changes in this output. Similarly fibres, which are silent in the isolated preparation, can be activated by stimulation of these nerves and their output changed by altering the frequency and stimulus strength.

The functions of these two ganglia have to be investigated before the control of the foregut can be clarified. Nevertheless it is helpful at this stage to suggest general ideas on the operation of the system.

Probably the foregut is normally held in a reeling position partly dependent on its mechanical characteristics and partly upon patterned output from the stomatogastric ganglia. This position can then be altered by information arising elsewhere in the nervous system (e.g. the supra-oesophageal and sub-oesophageal ganglia) and acting first on the commissural ganglia. Such changes probably occur when food is being passed to the mouth by the mouthparts. The commissural ganglia could then be further affected by sensory information from the mouth region (e.g. MPR 1, 2, 3) and also from the oesophagus and cardiac stomach (e.g. 'k' cells) as distortions were caused by food entry. This increased activity in the commissural ganglia could lead to activation of the stomatogastric ganglion via its stomatogastric nerve inputs. Increased activity of the stomatogastric ganglion would affect the p.s.n. sensory system and this input would feed back into the

system perhaps with progressively increasing effects (figure 2.1.17). The output of the ganglia would cause the cyclic activity seen in open preparations. Inhibition (i.e. slow down) of the system might be caused by information from mechanoreceptors in the pyloric region of the stomach reporting passage of food out of the foregut, cells on the cardiac stomach responding less as the stomach shrinks back to its normal (empty) shape, or too much if overfull. Also Bethe (see Bullock & Horridge, 1965) showed that a brainless crab will continue to feed beyond its capacity, therefore an inhibitory pathway from the brain to the stomatogastric system could operate. As the stomatogastric ganglion lies in the anterior aorta and the commissural ganglion has a well developed blood supply they could also receive excitatory and inhibitory stimuli from the blood. The cells in the ganglia may also be directly sensitive to movements of the foregut.

Of course such a general explanation says nothing of the precise mechanisms involved in the generation of central patterns and possible reflex activity, (i.e. does the sensory information just provide tonic stimulus to keep up the level of central activity or has any of it a precise role in timing?). Given the degree of sensory innervation and the environmental variation encountered (e.g. size, shape and hardness of food) it is probable that reflex mechanisms will be important in this

region (see appendix). Clearly the simplest sense organ to begin to investigate in these terms is MPR 1 and its involvement with the commissural and possibly the sub-oesophageal ganglia. The commissural ganglion is a particularly attractive target for physiological analysis at this time because it may be possible to study the activity of the cells in the same way as Maynard has done for the stomatogastric ganglion and to study the effects of sensory information on a central mechanism.

3.2.2. Other Aspects

The most straightforward question to be asked about the stomatogastric system is what is the effect of the motor supply to the muscles. Previous work on crustacean muscles has clearly demonstrated that the functions of a set of muscles cannot be deduced just by looking at the form of the motor output. The relationship between motor neurone activity and muscle function is rather complex. A problem which could be approached at the same time is the exact description of how the foregut moves. Again this does not appear to be simple although it is stereotyped.

Recently Winlow & Laverack (1969) have confirmed Miller's (1910) report that stimulation of the ventral nerve cord evokes peristalsis and anal opening in the hindgut. This would appear to link the anterior

stomatogastric system with some kind of command system to the last abdominal ganglion as there is no known supply to the gut from the other thoracic and abdominal ganglia. Similarly a command system is most likely between the supra- and sub-oesophageal ganglia and the commissural ganglia. This aspect will probably prove to be relatively simple for physiological analysis.

Osborne and I (1969) have recently suggested that monoamines may be involved in synaptic transmission in the decapod stomatogastric ganglion or between its motor neurones and the gut muscles. This links up with similar suggestions which have been made for the stomatogastric ganglia of insects (e.g. the ingluvial ganglion, Chanussot et. al., 1969). Such comparisons immediately introduce the question of possible neurosecretory mechanisms. The insect stomatogastric nervous system is intimately connected with the corpora cardiaca/corpora allata system and it is therefore necessary to ask if the crustacean system is linked to the endocrine system in any way. At this time there is no good evidence to suggest that it is.

To my mind the more interesting comparison with insect investigations comes not from looking ever deeper into the physiology of the system but from the possibility of correlating the electrophysiological analysis with behavioural observations and theories.

The most complete investigation is of course by Dethier and his collaborators on feeding in the blowfly Phormia. This study has extended over twenty years and has led to sophisticated theories on the control of feeding (Dethier and Gelperin, 1967). It has also been possible to study the chemosensory organs involved in some detail and a beginning has now been made on the electrophysiology of the stomatogastric nervous system (Gelperin, 1967). Some proposals have been made on the function of the cockroach stomatogastric nervous system, (Davy and Treherne, 1963) and electrophysiological investigations of sense organs and interneurons are in progress (Moulins, 1969). The decapods certainly have many advantages for electrophysiological investigations but is it possible to analyse feeding behaviour in a meaningful way?

Appendix 1. Proprioception in the Decapoda Crustacea

A.1.1. Definition

Pringle (1961) states that the term proprioceptor (from the Latin *proprio* = of our own motion) which was originally used by Sherrington, is part of a functional classification which needs not only knowledge of the sensory modality of the receptor but also of its role in the behaviour of the animal. The term was redefined by Lissman in 1950 to relate solely to the receptor. For Lissman proprioceptors were:

'Sense organs capable of registering continuously deformations (changes in length) and stresses (tensions, compressions) in the body. These can arise from the animal's own movements or may be due to its weight or to other external mechanical forces.'

Bullock and Horridge (1965) define the term simply as:

'Mechanoreceptors which normally signal movements or position of parts of the body'.

This is another definition concerned only with the receptor's response and not with its reflex function.

In the modern literature on crustacean receptors there is usually no clear statement of which of these two distinct types of definition (receptor response or receptor response plus reflex function) is being used. This is presumably because most workers in the field feel that

the distinction between proprioceptors and other receptors is clear.

Also the system allows receptors, which from their location appear to be proprioceptors, to be classified as proprioceptors. Apart from the unsatisfactory lack of clarity there is another serious disadvantage.

As Finlayson (1968) has most recently pointed out, some internally acting mechanoreceptors may be activating other responses, for example in endocrine systems, not short term postural or movement reflexes.

Therefore it is necessary to be clear about the definitions used in this thesis. Table A.1.1. sets out the nomenclature used. A receptor monitoring position or movements of parts of the main body of an animal is an Internal Mechanoreceptor. Internal refers to function not location; the group includes a few externally located receptor organs. An internal mechanoreceptor involved in short term reflexes of movement or position (which is not a statolith organ) is a Proprioceptor. I am therefore using a receptor response plus reflex function definition.

Any definition is going to be open to question by people with slightly different points of view. Explanations which may cover some questions are given in the notes for table A.1.1. It may be felt that the main trend of current opinion should be followed and my internal mechanoreceptors should be termed proprioceptors. Then it would be possible to subdivide into statolith proprioceptors, endocrine proprioceptors and

Table A.1.1.

Definition of a Proprioceptor

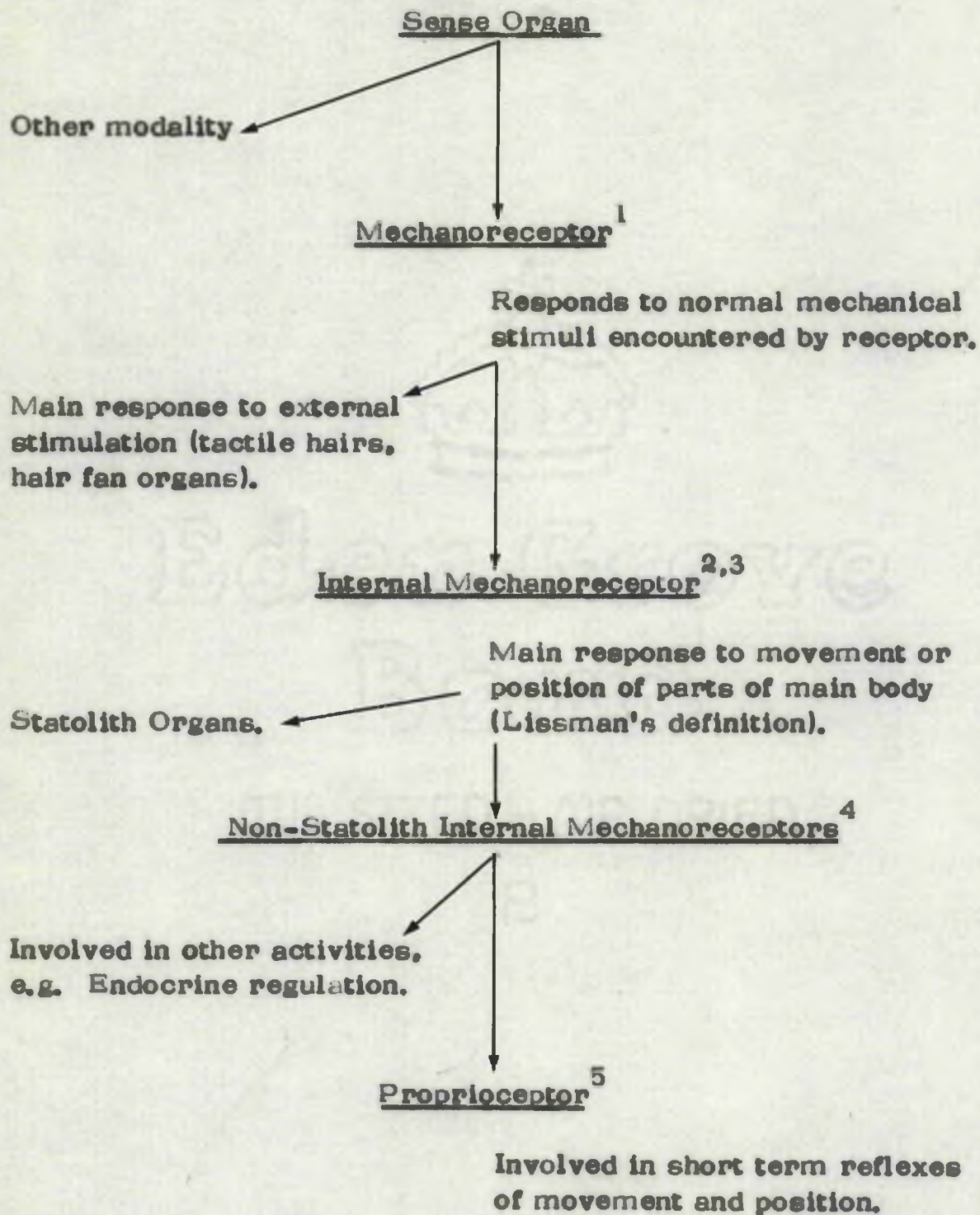


Table A.1.1.

Definition of a Proprioceptor

Notes

1. There is of course a possibility of a modality overlap.
2. It should be noted that Sherrington's original classification took account of location and source of stimulus. The location of the receptor is important in this definition. Most internal mechanoreceptors are internal but a few externally located mechanoreceptors (e.g. hair plates, Pringle, 1938) must respond almost exclusively to movement or position of the main body.
3. There is a possibility of an overlap of extero- and intero- responses in externally located receptors. Obviously hair fan organs will respond just as well when the animal moves its body through the water as when the water moves over its body. Surely the animal must be able to distinguish between these events by use of other sensory input and efferent copy mechanisms. The distinction between internal and external mechanoreceptors can therefore be made.
4. It is useful to exclude statolith organs because of their distinct structure and function. This is normal practice with many authors.

Table A.1.1.

Notes (cont'd.)

5. Clearly an internal mechanoreceptor could serve proprioceptive and other functions, but this is not necessarily true and therefore the distinction is justified.

movement and posture proprioceptors. This seems to me to make proprioceptor into a blunderbuss word (Gowers, 1962) and to lose the useful term Internal Mechanoreceptor. For the sake of argument here I stick to the nomenclature of table A.1.1.

A.1.2. Summary

Following Sherrington's work most studies on proprioceptors have been concentrated on vertebrates, particularly mammals, because of their intrinsic medical interest (Granit, 1955; 1963; Roberts, 1967). Yet, as in many other areas of biological research, fundamental studies are often more readily carried out on invertebrate preparations. This is mainly because the wide range of invertebrate types often provide examples with special advantages such as simplicity, size, disposition, or a limited number of neurones.

Although internal mechanoreceptors are known in Annelida and Mollusca (Laverack, 1968) most work on these receptors in invertebrates has so far been on arthropods (Finlayson, 1968). The decapod crustacea have provided many of these examples partly because of the large size of some species, the clear results which can be obtained by intra-vitam staining with methylene blue, and the ease of making electrophysiological recordings of nervous activity. Some of these studies, particularly of the abdominal MRO (Alexandrowicz, 1951, 1967) have produced results

of major importance. This large body of work on decapod proprioceptors and presumed proprioceptors has been extensively reviewed in recent years by Bullock and Horridge (1965) and again in the wider reviews of Finlayson (1968) and Laverack (1968).

There is no need to duplicate these reviews here. However, in Table A.1.2. I have attempted to summarise the development of the subject since Alexandrowicz described the MRO in 1951. In the table I have also attempted to show how the different anatomical types of receptor are arranged in the parts of the animal's body. It should be noted that the table is concerned with the following aspects of the work on decapod proprioceptors:

- a. Anatomy
- b. Fine Structure (to clarify anatomy).
- c. Operative Stimulus.
- d. Input to CNS.
- e. Reflex Effects.

No attempt is made to report studies of, for example, drug action or transducer mechanisms as these aspects are not relevant to the present experimental work.

The nomenclature of the internal mechanoreceptors in the decapod crustacea is mainly descriptive and due to numerous authors. It is

therefore confused. I have tried to order the nomenclature in terms of the operation of the receptor neurones.

I retain the uniterminal/multiterminal distinction because there may well be some difference in the mechanism by which the spike activity is generated in these two types. Therefore different mechanical forces may be needed to produce the same input to the CNS. I then distinguish three types of receptor organ.

The first of these are simple receptor organs in which the neurones send processes into cuticle or internal tissues but not on to a distinct strand or muscle. This category may include uniterminal neurone simple receptor organs (campaniform sensilla) or multiterminal neurone simple receptor organs ('k' cells of Orlov and Larimer and Kennedy). Strand receptor organs are the second category. In such receptor organs some type of strand directs the mechanical forces which operate on the neurones which innervate it. Again these receptors can be uniterminal (connective chordotonal organs of Howse 1968) or multiterminal (MPRI).

Finally there is the category of muscle receptor organs. In these organs the receptor neurones are connected with a muscle and it is therefore theoretically possible for the CNS to set the level of the activity of the neurones. These receptors include multiterminal neurone muscle receptor organs (MROs) and uniterminal neurone muscle

receptor organs (myochordotonal organs).

The whole system is therefore:

1. Simple Receptor Organs (no strand or muscle)

- a. Uniterminal - Campaniform sensilla
- b. Multiterminal - 'k' cells

2. Strand Receptor Organs

- a. Uniterminal - Connective Chordotonal Organs
- b. Multiterminal - MPRI

3. Muscle Receptor Organs

- a. Uniterminal - Myochordotonal Organs
- b. Multiterminal - Muscle Receptor Organs

A.1.3. Recent Developments

In the rest of this section several important developments which have taken place since the last reviews are discussed. These developments are:

- i. Clarac's reinvestigation of the leg myochordotonal organ in Carcinus.
- ii. Hartman and Boettiger's description of the disposition of movement and position receptors in a PD chordotonal organ.
- iii. Dendritic transmission of action potentials in bipolar

Table A.1.2.

Summary of Work on Proprioception in the Decapoda

Crustacea since 1950^{1,2}

1. Main Body

A. Abdomen

1.b. Multiterminal Neurons Simple Receptor Organs

- | | | |
|------|-------------------|---|
| 1967 | Pabet and Kennedy | Anatomy and physiology. ³
<u>Procambarus</u> . Receptor neurones with ramifying dendrites in ventral abdominal soft cuticle. Also collecting interneurones and reflex effects. See later in appendix for detailed discussion. |
|------|-------------------|---|

3.b. Multiterminal Neurons Muscle Receptor Organs⁴

- | | | |
|------|----------------------------|--|
| 1951 | Alexandrowicz | Anatomy. <u>Homarus</u> . <u>Palinurus</u> . Opened modern studies of proprioception in this group. |
| 1953 | Wiersma, Purenpan, Florey. | Physiology. <u>Procambarus</u> . MRO's shown to be phasic and tonic stretch receptors. |
| 1955 | Byzaguirre and Kuffler | Fundamental sensory physiology. <u>Procambarus</u> . <u>Homarus</u> . Start of the long series on this aspect. |
| 1966 | Fields | Reflex effects. <u>Procambarus</u> . Part of the classic series of papers by Kennedy and his |

associates: reviewed in detail in this appendix.

1967 Alexandrowicz

Review with notes for those foolish enough to indulge in physiology!

B. Thorax

3.b. Multiterminal Neurone Muscle Receptor Organs

I. Muscle Receptor Organs

1952 Alexandrowicz

Anatomy. Homarus, Palinurus.

1955 Kuffler and Eyzaguirre

Physiology. Homarus.
Effect of inhibitory axon.

1967 Alexandrowicz

Review.

ii. 'N' Cells

1952 Alexandrowicz

Anatomy. Homarus, Palinurus.

1961 Wiersma and Pilgrim

Anatomy and physiology.
Procambarus, Penaeus.

2. Appendages

A. Walking Legs

1.a. Uniterminal Neurone Simple Receptor Organs

(Campaniform Sensilla)

1968 Shelton and Laverack

Anatomy and physiology.
Homarus, Carcinus.

1. b. Multiterminal Neurone Simple Receptor Organs⁵

- | | | |
|------|---------------------------|-----------------------------|
| 1957 | Alexandrowicz and Whitear | Anatomy. <u>Homarus</u> . |
| 1967 | Alexandrowicz | Anatomy. <u>Palinurus</u> . |

2. a. Uniterminal Neurone Strand Receptor Organs⁶
(Chordotonal Organs)

- | | | |
|----------|---------------------------|--|
| 1954 | Burke | Anatomy and physiology of PD organ. <u>Carcinus</u> . |
| 1957 | Alexandrowicz and Whitear | Anatomy of TC and CB chordotonal organs. <u>Homarus</u> . TC not present in <u>Carcinus</u> or <u>Maia</u> . |
| 1958 | Alexandrowicz | TC not present in <u>Euphausiurus</u> . |
| 1959 | Wiersma and Boettiger | Physiology of PD. <u>Carcinus</u> . Characteristics of units. |
| 1962 | Whitear | Fine structure of most chordotonal organs. <u>Carcinus</u> . Hypothesis that paraciliary cell responds to strand elongation, ciliary to strand relaxation. |
| 1962b | Bush | Proprioceptive reflexes in PD and CP joints. <u>Carcinus</u> . |
| 1963 | Bush | Reflexes in PD and CP joints of a variety of species. |
| 1965a, b | Bush | Physiology of MC, CP and CB organs. <u>Carcinus</u> . |
| 1965c | Bush | Reflexes in all joints between coxa and dactyl. <u>Carcinus</u> . |

1966	Mendelson	Physiology. Impulse initiation in dendrites of PD neurones. <u>Callinectes</u> .
1967	Alexandrowicz	Anatomy. TC not present in <u>Pallinurus</u> .
1967	Hartman and Boettiger	Mechanical factors and cell responses in PD. <u>Cancer</u> .
1968	Clarac	Anatomy and physiology of IM organ. <u>Carcinus</u> .
1969	Clarac	Anatomy and physiology. BI organ in <u>Astacus</u> . Not present in <u>Carcinus</u> . Two IM receptors in <u>Astacus</u> .
1969a	Clarac, Wales, Laverack, Dando	Anatomy and physiology of most receptors in <u>Homarus</u> .

2.b. Multiterminal Neurone Strand Receptor Organs

(Innervated Elastic Strand Receptors, TC Complex)

1957	Alexandrowicz and Whitear	Anatomy. <u>Homarus</u> , <u>Carcinus</u> . Not present in <u>Mele</u> .
1958	Alexandrowicz	Anatomy. <u>Eupagurus</u> . Functional hypothesis.
1965	Whitear	Fine Structure. <u>Cardinus</u> .
1967	Alexandrowicz	Anatomy. <u>Pallinurus</u> .

3.a. Uniterminal Neurone Muscle Receptor Organs

(Myochoordotonal Organs)

1963	Cohen	Anatomy and physiology. <u>Cancer</u> .
1963	Cohen	Anatomy of receptor muscle. <u>Cancer</u> .

- | | | |
|----------|----------------|---|
| 1964 | Borai Raj | Physiology of receptor muscle. <u>Cancer</u> . |
| 1965 | Cohen | Reflex effects. <u>Cancer</u> . |
| 1967 | Cohen and Hess | Fine structure of receptor muscle. <u>Cancer</u> . |
| 1968a, b | Clarac | Anatomy and physiology. <u>Carcinus</u> . Reassessment. See discussion later in appendix. |
| 1969 | Clarac | Anatomy and physiology. <u>Astacus</u> . Only MCO 1 receptor. |

3.b. Multiterminal Neurone Muscle Receptor Organs.
(Muscle Receptor Organs, TC Complex)

- | | | |
|----------|---------------------------|---|
| 1957 | Alexandrowicz and Whitear | Anatomy. <u>Homarus</u> , <u>Carcinus</u> , <u>Male</u> . |
| 1958 | Alexandrowicz | Anatomy. <u>Eupaeurus</u> . Functional hypothesis. |
| 1965 | Whitear | Fine structure. <u>Carcinus</u> . |
| 1967 | Alexandrowicz | Anatomy. <u>Palinurus</u> . |
| 1968a, b | Bush, Roberts, Ripley | Operative stimulus, dendritic transmission to CNS without spikes, reflex effects. <u>Carcinus</u> . |

B. Claw 7.8

- | | | |
|------|------------------|---|
| 1962 | Bush | Reflex inhibition. <u>Carcinus</u> . |
| 1965 | Wilson and Davis | Nerve impulse patterns and reflex control. <u>Procambarus</u> . |

C. Antennule

1.a. Uniterminal Neurone Simple Receptor Organs

- 1964 Laverack Anatomy and physiology of neurones innervating the flagellae. Penulirus.

2.a. Uniterminal Neurone Strand Receptor Organs

- 1965 Wyse and Maynard Anatomy and physiology of joint receptors. Penulirus. One receptor responds to two axes of movement.
- 1967 Schöne and Schöne Integration of an antennular proprioceptive organ and statocyst. Penulirus.
- 1969 Schöne Anatomy and physiology of receptor at antennular base. Penulirus.

D. Antenna

1.a. Uniterminal Neurone Simple Receptor Organs

- 1967a, b Taylor Anatomy and physiology of an organ monitoring flagellum movements. Petrochirus.

2.a. Uniterminal Neurone Strand Receptor Organs

- 1969 Laverack and Dando Anatomy and physiology of main joint receptors. Homarus. One receptor spans two joints.

E. Mandible

1.b. Multiterminal Neurone Simple Receptor Organs

- | | | |
|------|--------------------|---|
| 1969 | Wales and Laverack | Anatomy and physiology.
<u>Homarus</u> . Neurones (in various nerves) with ramifying dendrites in epidermis. |
|------|--------------------|---|

2.a. Unterminal Neurone Strand Receptor Organs

- | | | |
|------|----------|--|
| 1969 | Laverack | Anatomy of receptor innervating palp. <u>Panulirus</u> . |
|------|----------|--|

2.b. Multiterminal Neurone Strand Receptor Organs

- | | | |
|------|--------------------------|---|
| 1968 | Laverack and Dando | Anatomy and physiology.
<u>Homarus</u> . |
| 1969 | Moulins, Dando, Laverack | Anatomy and physiology.
<u>Panulirus</u> , <u>Nephrops</u> . |

3.b. Multiterminal Neurone Muscle Receptor Organ

- | | | |
|------|--------------------|---|
| 1969 | Wales and Laverack | Anatomy and physiology.
<u>Homarus</u> . |
| 1969 | Laverack | Anatomy. <u>Panulirus</u> . |

F. Maxilla

1.b. Multiterminal Neurone Simple Receptor Organ

- | | | |
|------|---------|--|
| 1968 | Pasztor | Motor output controlling gill beater. <u>Procambarus</u> . |
| 1969 | Pasztor | Anatomy. Receptor associated with gill beater.
<u>Procambarus</u> . |

G. Maxillule

2.a. Uniterminal Neurone Strand Receptor Organ

1969 Laverack Anatomy. Panulirus,

H. Maxillipeds

2.a. Uniterminal Neurone Strand Receptor Organs

1969a Clarac, Wales, Laverack, Anatomy and physiology of
Dando receptors in 3rd maxilliped.
Homarus. No TC receptor,
no PD receptor.

2.b. Multiterminal Neurone Strand Receptor Organs

1969b Clarac, Wales, Laverack, Anatomy of receptors in TC
Dando region of Homarus. 2nd and
3rd maxillipeds. Not
present in 2nd maxilliped.

3.a. Uniterminal Neurone Muscle Receptor Organs

1969a, b Clarac, Wales, Laverack, No myochordotonal organ
Dando in 2nd or 3rd maxilliped.
Homarus.

3.b. Multiterminal Neurone Muscle Receptor Organs

1969b Clarac, Wales, Laverack, Anatomy of TC muscle
Dando receptor organs 2nd and
3rd maxillipeds. Homarus.

I. Swimmerets

1960b Hughes and Wiersma Central sequence responsible
for movements. Procambarus.

1964 Wiersma and Ikeda Command interneurons for
action of motor neurones.
Procambarus.

1968	Davis	Description of muscle system and motor output. Positions of sense organs noted. <u>Homarus.</u>
------	-------	---

J. Telson and Uropods

1964	Barth	Physiology. Not known anatomically. <u>Procambarus.</u>
------	-------	---

1967	Evoy and Kennedy	Overlap of command fibres for abdomen and tail positions. <u>Procambarus.</u>
------	------------------	---

K. Eyes

1968	Horridge	Central sequences control movement. No proprioceptors involved. <u>Carcinus.</u>
------	----------	--

3. Viscera

A. Gut

1.b. Multiterminal Neurone Simple Receptor Organs

1965	Larimer and Kennedy	Physiology. Unusual 'k' (Orlov) cells. <u>Procambarus.</u>
------	---------------------	--

1969	Dando and Laverack	Anatomy and physiology of posterior stomach nerve neurones, also reflex effects. <u>Cancer, Homarus.</u>
------	--------------------	--

1969	Moulins, Dando, Laverack	Anatomy and physiology of labrum receptors. <u>Nephrops.</u>
------	--------------------------	--

1969	Winlow and Laverack	Anatomy and physiology of hindgut receptors. <u>Nephrops, Homarus.</u>
------	---------------------	--

2.b. Multiterminal Neurone Strand Receptor Organs

- | | | |
|------|--------------------|--|
| 1968 | Laverack and Dando | Mandibular receptors also respond to oesophageal movements. <u>Homarus</u> . |
| 1969 | Laverack | Mandibular receptors also respond to oesophageal movements. <u>Panulirus</u> . |

B. Nervous System

1.b. Multiterminal Neurone Simple Receptor Organs

- | | | |
|-------|-----------------------|--|
| 1926 | Orlov | Anatomy. Presumed proprioceptor neurones with ramifications on oesophageal connective sheath. <u>Astacus</u> . |
| 1960a | Hughes and Wiersma | Physiological results indicating receptors on abdominal cord. <u>Procambarus</u> . |
| 1966 | Kennedy, Evoy, Fields | Physiological results indicating receptors on abdominal cord. <u>Procambarus</u> . |

Notes for Table A.1.2.

1. Work concerns mainly Reptantia, Macrura and Brachyura.
Generic names of species used for major part of each investigation are given.
2. Internal mechanoreceptors which are thought to be proprioceptors are included for the sake of completeness.
3. Physiology is normally used here to cover input to CNS and operative stimulus. Other subjects such as reflex effects are cited where applicable.
4. Of course only a few of the hundreds of papers on the MRO can be given.
5. Cells, some of which are multipolar, were described in the leg nerves in both of these papers. It seems probable that these cells are internal mechanoreceptors and also as no receptor structures were found that these neurones are in the l.b. group.
6. Some of the connective chordotonal organs in the appendages are attached to muscles. Therefore they can be set by the CNS - but only to imposed forces. Such receptors are in group 2 not 3.
7. Where little information on internal mechanoreceptors is

available but something is known of the motor system this information is given.

8. For the most part the claw bearing appendages have similar receptors to the walking legs. There is, however, no description of the receptor monitoring the claw. This is of some interest in species where there are large differences in the structure and function of the two large claws.

receptor neurones, and conduction of graded potentials by the dendrites of the T/C receptors in Carcinus.

- iv. Finally a more extensive review is given of the work of Kennedy and his associates on the control of abdominal position in the crayfish as this is a model for any investigation of proprioceptors and their role in the behaviour of an animal.

- i. The Myochoordotonal Organ

In 1934 Barth described a complex of peripheral neurones and associated structures in the I-M region of a number of decapod crustacea. Cohen (1963, 1965) reinvestigated this receptor in Cancer and confirmed its association with a specialised muscle. He showed that the receptor he described contained unidirectional movement and position fibres responding to M-C joint extension and flexion. These units showed marked range fractionation over the total arc of joint movement. He also showed that the sensory input from the receptor could be modified by stimulating the single motor axon innervating the associated receptor muscle. Contraction of the muscle reproduces the effect on the sense cells of extending the MC joint. The cells responding to MC joint extension are thus facilitated and those responding to flexion of the MC joint are depressed. The receptor can therefore be set by central

commands to the associated muscle which makes it a most complex and interesting system.

Curiously no marked malfunction of the MC articulation in walking followed removal of the organ; instead a depression of total locomotor activity followed removal of several receptors. Cohen postulated that the receptor input could therefore be setting the level of central excitability.

Clarac (1968, 1969a, b) who was interested in the relationship of this system to the vertebrate muscle spindle had difficulty in reconciling Cohen's description of the MCO with Barth's work and his own anatomical results in Carcinus. He was therefore forced to reinvestigate the anatomy of the whole region and the physiology of the receptors found there. Table A.1.3. summarises his anatomical findings and their relations to previous work. Table A.1.4. summarises his physiological findings.

This receptor system is of great interest as it is even more complex than Cohen's work suggested. The major question now to be resolved is what is its role in the locomotor activity of the decapoda crustacea. In this respect it should be noted that Clarac has recently confirmed that a similar but anatomically different system exists in Reptantian species (Clarac, 1969), and also that Wales and Laverack (1969)

Table A.1.3.

Proprioceptor Anatomy of the Ischio-Meropodite Region in
Legs of the Crab *Carcinus mediterraneus* C.

- I. Three groups of proprioceptive bipolar neurons were found at the ischio-meropodite joint of all walking legs and chelipeds of the crab *Carcinus mediterraneus* C.

Two of these receptors are connected with the proximal head of the accessory flexor muscle (AFM) of the carpopodite:

- a) The first (Barth's organ) is situated near the postaxial wall of the meropodite; it is associated with the AFM by an elastic membrane.
- b) The second is in the preaxial part of the ischiopodite above muscle fibres of the retractor of the meropodite; it is associated with the dorsal part of the AFM by an elastic strand;
- c) The third receptor consists of twenty sensory cells in the post-axial region of the ischiopodite attached to the tendon of the meropodite retractor muscle, its strand stays in the same segment.

- II. Comparison with previous research permits all structures connected with AFM to be considered as components of the myochordotonal organ (MCO), and the third receptor to be

named the IM (Ischiomeropodite) chordotonal organ.

The organ described by Barth should be equated with MCO 1 in Carcinus, subdivided, usually into two groups: the main organ ("Hauptorgane") and the proximal organ ("Proximalorgane"). The receptor studied by Cohen was the second receptor (MCO 2).

Table A.1.4.

Physiology of Proprioceptors in IM Region

- I. The two receptors connected with the accessory flexor muscle (AFM) were stimulated by mero-carpopodite (M-C) joint movement, while the activity of the IM cellular group depended only on ischio-meropodite (I-M) joint displacement.
- II. MCO presented a great variety in cellular discharge pattern depending on whether the units were from the first or the second MCO receptor.
 - a) Unidirectional movement and position fibres for stretch or release of the AFM were recorded from the first MCO receptor (Barth's organ).
 - b) The nerve from the second MCO receptor contained chiefly fibres sensitive to flexion of the M-C joint.
- III. The chordotonal organ IM which anatomically is similar to the chordotonal organs MC 1 and CP 1, showed a maximal discharge during protraction of the meropodite, when its elastic strand was released that is like MC 2 and CP 2.

have found a receptor with certain similarities to this system in the mandibles of Homarus vulgaris. Also it is known that no such receptor occurs in the antennule (Wyse and Maynard, 1965) or in the 2nd and 3rd maxillipeds (Clarac, Wales, Laverack, Dando, 1969ab) of Reptantians.

ii. Functional Organisation of Chordotonal Organs.

After Whitear's (1960, 1962) description of the fine structure of the leg chordotonal organs of Cardius efforts were made to relate the structure of the dendrites of the receptor cells to their physiological properties. The theory suggested by Whitear was that the paraciliary cell responded to elongation of the strand and the ciliary cell responded to strand relaxation. This theory finally proved untenable when Bush (1965b) showed that although the paired segments of the CB organ are both ciliary the CB organ responds to both elongation and relaxation of the receptor strand.

Unfortunately it had been forgotten that Wiersma and Boettiger (1959) had proposed a simpler theory to explain some of the receptor properties of the chordotonal organs. They attempted to relate the disposition of the cell bodies on the receptor strand to the neurones' responses. This idea was again put forward by Wyse and Maynard (1965) in their study of the antennular chordotonal organs of Panulirus.

In a recent investigation Hartman and Boettiger (1967) showed that the receptor properties of the cells in the PD organ of Cancer could in fact be related to the disposition of the cells on the receptor strand. Figure A.1.1. summarises their findings. Clearly this work has set new standards for investigations of decapod proprioceptors, particularly by its emphasis on the constancy of the peripheral neuronal architecture in the PD organ. Nevertheless we are still a long way from a mechanical explanation of the physiological properties of the neurones in a simple chordotonal organ.

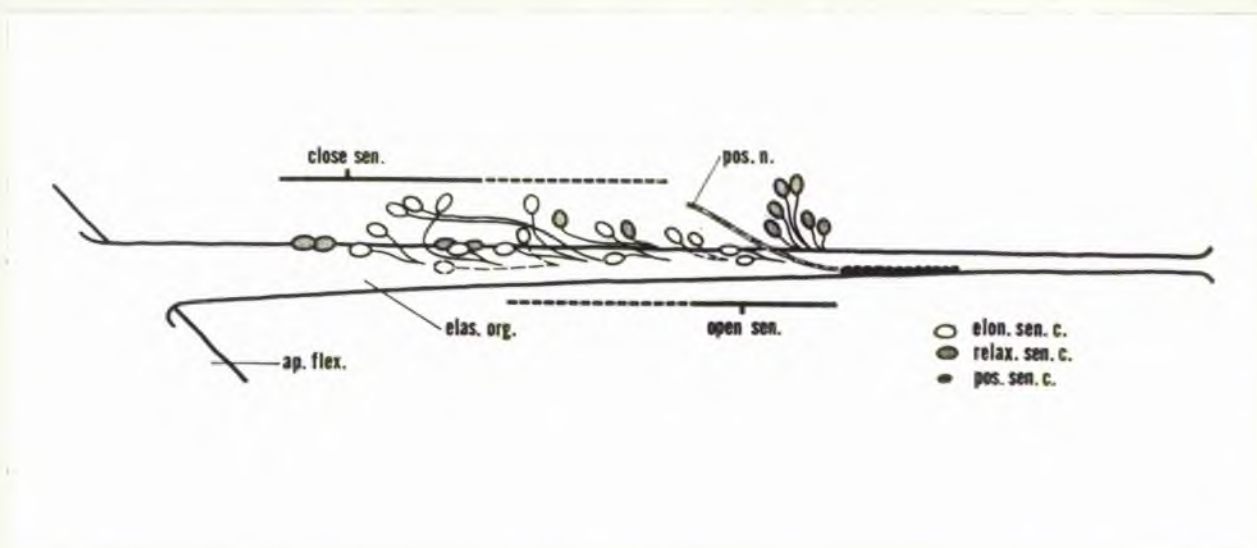
iii. Types of Conduction

The conventional doctrine often conveyed to students is that dendrites conduct graded potentials to the cell where action potentials are set up and conducted along the axon to the next point in the chain. Yet, as Bullock and Horridge (1965) have pointed out, dendrite and axon are anatomical terms and should convey no physiological connotations.

This simple point has been nicely illustrated by recent work on mechanoreceptors in the decapods. Mellon and Kennedy (1964) were first to present evidence that the dendrites of cutaneous mechanoreceptors in this group conducted action potentials. Their work was supported by evidence gained from Mendelson's work on cells in the PD chordotonal organ of Callinectes (1966). Then Hartman and Boettiger

FIGURE A.1.1.

Diagrammatic drawing showing the organization of the PD organ. The anterior surface faces the reader and dorsal is upwards. Movement axons have not been drawn for clarity, ap. flex, apodeme of flexor; close sen., movement neurons (ESC and RSC) with close-position-sensitivity; open sen., movement neurons (ESC and RSC) with open-position-sensitivity; elas. org., elastic organ or strand; pos. n., position nerves or bundle; elon. sen. c., elongation sensitive cells; relax. sen. c., relaxation sensitive cells; pos. sen. c., position sensitive cells. Dashed lines indicate movement neurons which are equally sensitive to any position.



(1967) showed that it was possible to record extracellularly from two points on the dendrites of neurones in the PD organ of Cancer and to determine the conduction velocity of the impulse.

Similarly Larimer and Kennedy (1966) and Pabst and Kennedy (1967) showed that in multiterminal neurones impulses are conducted in the dendrites. Here also it was possible to determine conduction velocities, and something of the mechanism whereby the widespread terminals are activated. It is difficult to believe that the 0.5 cms long dendrites in the myochordotonal (MCO2) organ and the 1.5 cms dendrites of the p.s.n. neurones do not carry action potentials. Indeed it seems possible that this is a general feature of decapod mechanoreceptors.

As a corrective to such generalisations it should be noted that Bush, Ripley and Roberts (1968a, b) demonstrated that the dendrites of certain neurones innervating receptors in the T/C complex of Carcinus normally conduct only graded potentials into the CNS and that this information is sufficient to produce reflex activity. This physiological work provides some explanation of the odd anatomical findings of the large diameter innervating processes in Alexandrowicz and Whitear's original description.

iv. Control of abdominal position in the crayfish

The investigations discussed here are all by Kennedy and his

associates. The papers are listed in Table A.1.5. The work demonstrated the function of the slow tonic MRO and one other sensory system in the control of abdominal posture in the crayfish. It also showed the relationship of the peripherally originating MRO information to the centrally generated command information within the total control system .

Initially the major effort was devoted to discovering the properties of the neuromuscular system in the abdomen. It was found that there were two distinct anatomical and functional systems of muscles. To paraphrase Kennedy, Evoy, Fields (1966):

'Between 80 and 90 per cent of the segmental abdominal musculature (the deep twitch system of extensors and flexors) and over 60 per cent of the motor neurones supplying it, have been invested in a stereotyped, special behaviour (the escape tail flip) performed only in relatively rare emergencies'.

They took advantage of this by leaving aside the large component and concentrating their efforts on the rest. That is the superficial extensors and flexors concerned with posture of the abdomen. The fact that these muscles are supplied by a small number of motor neurones which can be identified by spike size considerably aided their investigations.

Table A.1.5.

Papers on the Control of the Position of the Abdomen in Crayfish

1965	Fields and Kennedy	Functional role of MRO.
1966	Kennedy and Takeda	Reflex control of abdominal flexor muscles. I. Twitch system. II. Tonic system.
1966	Kennedy, Evoy and Fields	Unit basis of some crustacean reflexes.
1966	Fields	Proprioceptive control of posture in crayfish abdomen.
1966	Kennedy, Evoy, Hanawalt	Release of co-ordinated behaviour in crayfish by single central neurones.
1967	Fields, Evoy, Kennedy	Reflex role played by efferent control of an invertebrate stretch receptor.
1967	Evoy, Kennedy, Wilson	Discharge patterns of neurones supplying tonic abdomen flexor muscles.
1967	Pabst and Kennedy	Cutaneous mechanoreceptor influencing motor output in crayfish abdomen.
1967	Evoy, Kennedy	Central nervous organisation underlying control of antagonistic muscles in crayfish. I. Types of command fibres. II. Coding of position by command fibres.

A. Command Fibres

Before going on to consider in detail the role of the sense organs in the control system it is necessary to deal with three of the later papers on command fibres. These investigations are summarised in table A.1.6. One of their other major conclusions is of interest here :

'Single interneurones, or several in combination, thus provide entirely adequate channels for the production of complex co-ordinated movements. Though proprioceptive feedback from the periphery may be used to stabilize or amplify some movements, it is entirely unnecessary for their co-ordination or for their temporal regulation'.

The sense organs we are dealing with therefore function in this minor role. The basic pattern is generated centrally (though clearly this is determined by sensory input patterns, see table A.1.6., section 4).

B. The Tonic Motor System

Figure A.1.2. shows the layout of the muscles and ganglia in the crayfish abdomen. Table A.1.7. summarises the information on the neuromuscular system of the superficial extensor and flexor sets of muscles. Kennedy, Evoy and Fields (1965) conclude:

'The neuromuscular apparatus is highly complex. Regions of single muscles have an internal heterogeneity which we, at least, had

Table A.1.6.

Command Fibres in the Control of the Crayfish Abdomen

1. A limited number of "command" interneurons mediate the release of co-ordinated ganglionic output to antagonistic postural muscles in the crayfish abdomen. Graded contractions of these muscles are regulated by the frequency of discharge in several motoneurons, whose own discharge is in turn controlled by the level of activity in command fibres.
2. Different command fibres having a similar effect (i.e. flexion or extension) vary in the relative effectiveness with which they excite specific motoneurons. This selectivity is responsible for different rates of tension development.
3. Command fibres connect with the postural motor system in each segment in a bilaterally symmetrical fashion. Many of the same command elements also have unilateral or reciprocal effects on other motor systems, especially on those which control the swimmerets and uropods.
4. The reciprocity between flexors and extensors is inherent in the ganglionic organization. Activation or modulation of this ganglionic centre is accomplished by the command interneurons, which in turn are activated by appropriate combinations of

sensory inputs. A wide variety of postural adjustments may thus be controlled by a limited number of central neurons that make complex but specific output connections.

5. Single central interneurons that produce flexion or extension in the crayfish abdomen act in a co-ordinated fashion upon several ganglia. Each of several elements evoking a similar type of movement has a unique distribution of output to ganglionic centres: thus one of them may produce primarily rostral extension or flexion, another a primarily caudal movement, still another a more general one. Each motor command therefore appears to code for a specific abdominal geometry. Some units produce complex, cyclic motor outflow to postural muscles of the abdomen, or to the appendages.

FIGURE A.1.2.

Diagrammatic representation of a lateral view of the tonic muscles, their innervation, and the exoskeleton of three abdominal segments in the crayfish. E, superficial extensors; F, superficial flexors; MRO, tonic muscle receptor organ; R₁₁₁, superficial branch of the third root; R₁₁, second root. Heavily inked portions of exoskeleton are rigid, lightly inked ones flexible. The circles at the intersegmental junctions mark the approximate pivot joints to flexion and extension. The important feature of the arrangement is that flexor activity in one segment bends the anterior joint of that segment, stretching the MRO belonging to the next segment ahead.

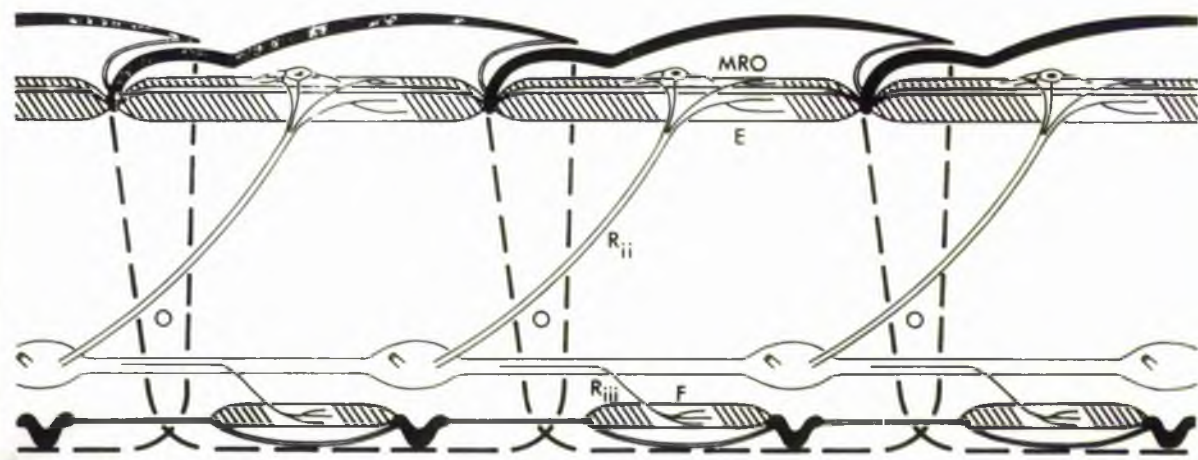


Table A.1.7.

Properties of slow flexor and extensor motor neurones

	Action on muscle	% muscle fibres innervated	Temporal properties	Average frequency
Flexor				
1	Small ejp's	45 (mostly medial)	Some facilitation	12/sec.
2	Usually small ejp's	27 (mostly medial)	Some facilitation	17/sec.
3	Large ejp's	86	Variable, usually some facilitation	12/sec.
4	Large, fast-rising ejp's	55 (mostly lateral)	Variable, usually some facilitation	10/sec.
5	Hyperpolarizing ijp's	40 (mostly medial)	Facilitation	0
6	Small ejp's	53 (mostly lateral)	Marked facilitation	Variable, usually 0
Extensor				
1	ejp's	30-60, mostly lateral	Facilitation	1-2/sec.
2	ejp's	Nearly 100	Facilitation	0
3	ejp's	Unknown	Facilitation	0
4	ejp's	30-60;	Facilitation	2/sec.
5	Large ejp's	c. 30, mostly lateral	Facilitation	0
6	Hyperpolarizing ijp's	c. 40	Facilitation	1-2/sec.

not expected; and different elements within them may be called into action for reflexes differing in speed of execution or source of stimulus. Finally, although sets of motor axons do not appear to be connected with great precision to muscle fibres, the elements appear to possess considerable individual functional specificity. The concept of a motor neurone "pool" is thus not very helpful in describing the system'.

The interesting recent developments in arthropod neuromuscular investigations form a different story which will not be dealt with here.

C. Sensory Systems

a. The Extensors and the Tonic MRO

By recording the central effects of MRO stimulations it was shown that the medial phasic MRO was probably associated with the phasic motor system. It had no demonstrable effect on the tonic muscle system. On the other hand the activity of the tonic MRO was shown to be intimately connected with the functions of the tonic extensor neuromuscular system. I am therefore only concerned with this receptor.

It was then shown that the receptor muscle RM, shares a motor neurone with the functional extensor muscles. Graded stimuli were applied to the dorsal nerve (part of root two, rii figure A.1.2.) and the receptor discharge monitored. It was possible to correlate sudden

increases in sensory output with the appearance of junctional potentials in the slow extensor fibres as the intensity of shocks to the nerve was increased. In most cases the receptor muscle is only innervated by axon 4 of the motor neurone system.

Then returning to the central effects of stimulation of the receptor it was shown that only one of the motor neurones to the functional extensors was activated by the receptor input. The axon was identified as No. 2. This motor neurone innervates over 90 per cent of the superficial extensor muscle fibres and tends to produce large junctional potentials in most of them (table A.1.7.) It is therefore a highly effective route for reflex excitation of the muscle system. The input from the stretch receptor to this axon shows a dramatic summation with tactile input. The relationship between MRO receptor discharge and motor axon 2 discharge is linear. In addition a weak inhibitory effect of MRO discharge on the smallest axon 1 was demonstrated. Axon 1 may occasionally innervate the receptor muscle but axon 2 certainly does not. These relationships between the MRO and the extensor motor neurones are given in table A.1.8.

The discharge of the tonic MRO was found to have a limited segmental effect. Besides the excitation of axon 2 it only excites the inhibitory accessory nerve to itself. There is no effect on the contra-

Table A.1.8.

Summary of extensor and MRO efferent connections

<u>Axon</u>	<u>MRO Effects</u>	<u>Innervation</u>
1	Inhibited	Extensors, MRO(?) (excites)
2	Excited	Extensors only (excites)
3	None	Extensors only (excites)
4	None	Extensors, MRO (excites)
5	None	Extensors only (inhibits)
6	None	Extensors only (excites)
Accessory nerve	Excited	MRO dendrites and soma (inhibits)

lateral axon 2. Also the effect on axon 2 is virtually segment specific being 10 times greater in the receptor segment than in the next anterior or posterior segment. The intersegmental effect on the accessory nerve is strikingly different, the next anterior segment accessory nerve being affected to a greater extent.

It was further found that in natural and experimental situations different types of command fibre can activate only motor axons to functional muscles (not axon 4) or functional muscles plus axon 4. The two different command fibre types would clearly have a different effect on reafferent excitation of axon 2. Similarly natural and experimental activation of the inhibitory accessory nerve to the receptor muscle were demonstrated. This leads to a reduction in axon 2 discharge.

Fields (1967) studied the position of the abdomen and MRO discharges occurring at the same time. He found that;

- (a) receptor output varied widely with small changes in position and
- (b) conversely there was often no receptor output when the abdomen changed its degree of flexion extensively.

Receptor output sometimes actually increased when the segment shortened; the exact opposite of what would be expected from the anatomy. Most noticeably it was repeatedly observed that if the abdomen was manually flexed from a set position it returned to that position, often

with remarkable precision. The slow MRO discharge increased when the tail was manually flexed and was progressively silenced as the initial position was reapproached. Clearly the MRO is not a simple length detector. To quote Fields:

'The analysis of the innervation pattern and reflex effects of the tonic MRO indicates that the following sequence of events takes place in naturally occurring extension of the tail. When the segmental slow extensor motoneurone(s) which supply RM1 are activated, the muscle of the tonic MRO contracts and the receptor discharge increases; this in turn feeds back as excitation to the most widely distributed of the slow motoneurones. As the extensor muscles shorten, tension in RM1 decreases, resulting in a decreased frequency of firing in the sensory neurone. The validity of this postulated sequence is supported by results showing that tactile stimulation may cause a transient rise in the frequency of receptor discharge which in turn evokes motoneurone discharge (Fields & Kennedy, 1965). Since the impulse frequency of the MRO is a linear function of tension, which is in turn directly related to the initial length of RM 1, the magnitude of the sensory discharge fed back in response to motoneurone activity will depend on the initial length of RM1.'

Fields' diagrammatic representation of the function of the tonic MRO

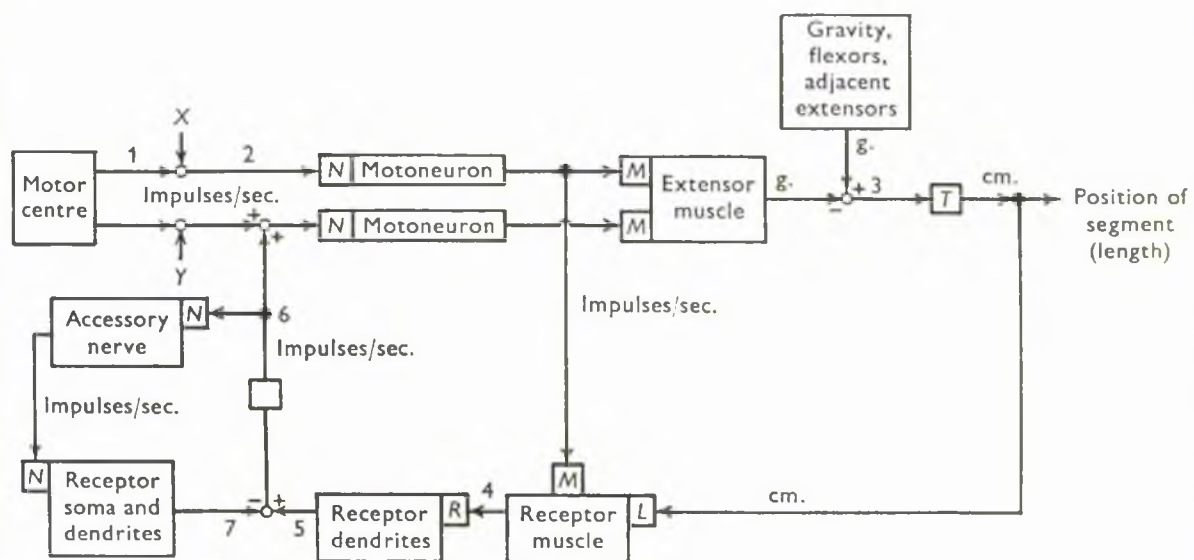
is shown in figure A.1.3. His comments on this model are worth quoting in some detail:

'Presynaptic drive to the slow extensor motoneurone(s) that supply RMI determines the set point. The output, tail position (in this case, lengthening of the reference segment), represents a balance between the various inputs including segmental motoneurone activity (negative, i.e. shorten the reference segment) and the opposing forces of flexor contraction, gravity and contraction of extensors in adjacent segments (positive, i.e. lengthen the reference segment). The stretch receptor acts as an error detector which responds to differences between the set point (at which its output is zero) and the actual tail position; the magnitude of this error is, of course, given by the impulse frequency of the MRO. MRO discharge activates one of the extensor motoneurones, resulting in shortening of the segment and reduction of the error. By altering excitatory input to the motoneurone supplying RMI the set point can be varied, allowing the servo loop to operate over a wide range of abdominal positions. Such a system enables the crayfish to adjust the force of slow extensor contraction to any level that might be required to compensate for changes in load or muscle power (i.e. resulting from fatigue or contraction of synergists).'

'The finding that the excitation fed back to the slow extensor motoneurones via MRO discharge does not affect the motoneurones supplying

FIGURE A.1.3.

Block diagram of the servo control system for regulation of tonic contraction. Large rectangles represent structures named within; small squares represent transducing functions; N, neuro-neural synapse; M, neuromuscular junction; T, converts tension to length change (i.e. series and parallel visco-elastic properties of the slow extensor muscles); L, converts length change to tension (visco-elastic properties of RM 1); R, converts dendritic deformation to membrane depolarization (receptor potentials). Open circles represent summing points and closed circles represent pick-off points for feedback loops. (1) Reference input, related to 'desired' position (i.e. set point); (2) actuating signal (excitatory input to motoneurone supplying RM 1); (3) controlled variable (net tension in extensors); (4) by altering the mechanical properties of RM 1, i.e. its length-tension curve, activity in the motor nerve to RM 1 determines the discharge rate of the MRO at any given length. RM 1 length equals segment length. If segment length is greater than 'desired', RM 1 tension produces a generator potential (5) which exceeds threshold for MRO discharge, the error signal (6); (7) inhibitory junctional potential set up by accessory nerve activity.



RM1 is of theoretical importance, since if MRO output caused contraction of RM1 the error signal would be distorted. Further, this type of positive feedback, although diminished by concomitant extensor contraction tending to unload the receptor, could lead to instability of the servo mechanism.'

'It is significant that the motoneurone activated by the tonic MRO has the lowest threshold to tactile stimulation and apparently supplies every superficial extensor muscle fibre except the receptor muscle. Among the consequences of this arrangement are: first, that the wide distribution and greater excitability of the motoneurone activated by the MRO increases the gain of the servo loop without sacrificing stability; and second, that the arrangement allows filtering of 'noise', since transient minor input will cause only a transient shortening of the segment and will not activate the servo loop. Finally, the tonic extensor motoneurones can serve an 'arousal' function, since tactile stimulation almost anywhere on the exoskeleton will excite them. If the servo loop is then brought into action by higher centres, the MRO input will summate with the tactile input either centrally or at the neuromuscular junction.'

Finally, Fields, Evoy, Kennedy (1967) made some statements about the accessory nerve reflex and the total control system.

'Central initiation of inhibitory outflow to the MRO via the accessory

nerve has been experimentally produced by stimulation of interneurons and of the MRO afferent itself. These reflexes tend to inactivate the servo loop in the affected segment, and thereby decrease the resistance to centrally evoked flexion. Since any known central or segmental input producing flexion will reciprocally inhibit extensor motoneurons, the resistance reflex loop in that segment is broken and there is little need for self-inhibition of the MRO. The results show, however, that the most powerful inhibition produced by accessory nerve discharge is exerted upon the next anterior ganglion. Indeed, the innervation of antagonist muscles is such (figure A.1.2) that active flexion in a given segment provides MRO input in the next anterior ganglion and hence gives a maximal accessory nerve reflex two ganglia away. At this point the central drive on the axon driven by the MRO (no. 2) will be reduced. Flexion initiated in any posterior ganglion by central command - or by imposed forces - will thus produce a flexor bias anteriorly, and probably would result in overall changes in conformation that follow the local change.

Of the two control systems studied that affect extensor muscles, one mediates a set position of the abdomen through the adjustment of a length-specific reference system and the other evokes a wide range of movements that are superimposed upon this. The separation of central

pathways for these two effects allows temporary changes in position to be interpolated into the posture set by the MRO and, at the same time, permits new postures to be adopted through alterations in MRO excitability. The multisegmental nature of the accessory nerve reflex provides overall co-ordination by temporarily shutting off the receptor signal in anterior segments. Since axon 4, which innervates the receptor muscle, is not affected by this reflex, the centrally determined setpoint is preserved. Various interruptions in the posture maintained by the MRO servo are thus possible, whether they are mediated by unshared motoneurons or by the anteriorly directed cascade of accessory nerve activity. Following such relatively transient changes the abdomen will resume the initial conformation set by efferent discharge to the MRO'.

To summarise, the MRO functions to correct imbalances between positions set by the CNS and the position achieved in the environment. For instance if the crayfish moves from water into air the same set level of activity in the shared motoneurone will produce a shortening which lags behind the tension developed in the receptor muscle. The resulting MRO discharge will therefore supply more tension in the functional muscle via axon 2 until the set position is reached.

b. The Flexors and the Ventral Cutaneous Mechanoreceptors

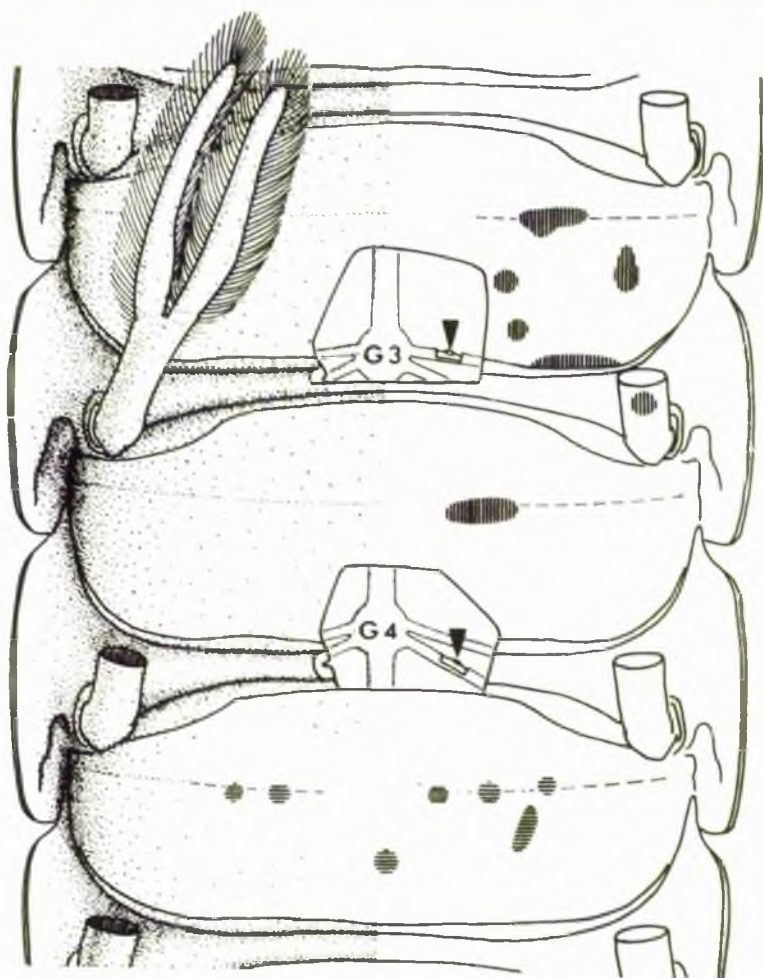
One sensory system has so far been found to affect the motor supply to the flexor muscles of the postural system. These sensory cells have been described by Pabst and Kennedy (1967). Figure A.1.4. shows the location of these cells. In the first and second root of all abdominal ganglia several somata (0-6) were usually located within a few mm. of the ganglion. The cells were 50-70 μ m by 20-45 μ m. Approximately 80 per cent were bipolar and the rest tripolar. The dendritic processes of these cells spread over a wide area.

The cells respond to mechanical stimulation of widely spaced areas of cuticle (figure A.1.4.). The terminals of the dendritic ramifications were shown to be invaded by axon reflex and the dendritic conduction time to be 0.8m/sec.

Stimulation of these receptors by gentle pressure on the soft cuticle of the abdomen causes suppression of the spontaneous motor output that goes to the slow flexor muscles via the superficial branch of the 3rd root. The receptors produce an even stronger suppression of the motor output to the swimmeret muscles causing immediate relaxation of the appendages. The effect on the motor system of natural stimulation was shown in adjacent ipsilateral segments and to a lesser extent contralaterally.

FIGURE A.L.4

View of a portion of the crayfish abdomen, showing ganglia 3 and 4 exposed with their first and second roots. The sensory fields of the two cells indicated by the arrows in the first root of ganglion 3 and the second root of ganglion 4 are shown by the vertically and horizontally striped areas, respectively. The dotted lines show the attachments of the superficial flexor muscles.



The intersegmental reflex effects allowed the convergence of these afferents upon single multisegmental interneurons to be examined and also an attempt to be made to determine whether activity in these interneurons is directly responsible for commands to the motor system. Again the conclusions are worth quoting in detail.

'The convergence of input from these ventrally located cutaneous mechanoreceptors upon central interneurons obeys in a general way the rules established for other sensory modalities in the same organism (Wiersma, 1958; Wiersma and Hughes, 1961). Single interneurons collect input from homologous regions of a number of adjacent appendages; and it is possible to specify individual interneurons uniquely in terms of the combination of peripheral areas they represent and their locations in the central connective. Like interneurons belonging to other systems, these show some tendency to receive more than one modality. In particular, it is not uncommon for hair receptors and pressure receptors from the pleural plate to share central connections with the cuticular fibers. The number of interneurons involved in the central representation of the cuticular input suggests that it is a significant one.'

'The experiments afforded us an opportunity to inspect the sensory fields of such interneurons and their reflex effects in the same experi-

mental setting. Stimulation of single fibers had previously been shown (Kennedy, Evoy and Hanawalt, 1966) to produce complex, reciprocal motor outputs to postural muscles in the abdominal segment. Since natural stimulation of the receptors under study here produced strong reflex effects on that system, it was natural to attempt to demonstrate motor connections for the same interneurons from which we recorded. In fact, nearly all of the 21 units in our sample were obtained by the procedure of stripping fine filaments of nerve from a connective, stimulating them, and further analyzing those which produced reflex suppression of motor outflow to the slow flexor muscles. We encountered little success in our few attempts to find these interneurons by dissecting bundles and exploring the receptive fields of units in them one after another. In every case where an interneuron received powerful input from the ventral cuticle in the region of the slow flexor muscles, electrical stimulation of that unit at 75 per sec produced effective suppression in one or more abdominal segments. We were not able, in these experiments, to correlate the segmental distribution of motor outflow with that for input. However it was observed that most such neurons produced effects in at least two adjacent segments. Our experiments do not show that these interneurons act directly upon motoneurons; they may be separated from the final pathway to muscle

by one or more synapses. It is clear, however, that interneurons spanning several segments in the abdominal region of the central nervous system are located directly on the pathway from receptor to motoneuron, and they serve to spread the influence of a regionally localized stimulus to the entire responding system.'

A.1.4. Conclusions

It seems probable to me that we shall soon have reached the end of the first stage of the investigations in this subject. We shall know the anatomy, operative stimulus and input characteristics of the major proprioceptors in a few of the decapods. Kennedy and his associates have indicated the further investigations which must follow before a reasonable understanding of the role of these organs will be obtained. Behaviour, neuromuscular systems, interneurons must all be analysed. Perhaps the specific gap in the work discussed in the last sub-section, the relationship of sensory collecting interneurons to premotor interneurons, will prove to be the most interesting and difficult general problem in these further studies.

Finally it is necessary to return to the question of why animals with such important command systems need proprioceptors. The answer is of course that in many instances the effect of the central command will be distorted in the operating system. For example Wilson (1968) thinks that in the locust flight system the effect of

proprioceptive input is to determine the most efficient wingbeat frequency in relation to the animal's size. The crayfish abdomen position needs to be more closely controlled and therefore the proprioceptors are more complex and important. This is presumably also true of the walking legs whose operation must be closely matched to the substrate conditions.

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